JEB®

Journal of Environmental Biology

p-ISSN: 0254-8704 • e-ISSN: 2394-0379 • CODEN: JEBIDP

Journal website : www.jeb.co.in ★ **E-mail :** editor@jeb.co.in

Triveni Enterprises (Educational Services Set-up)

Original Research

DOI : http://doi.org/10.22438/jeb/43/6/MRN-1740

Isolation and bioactive potentials of *Streptomyces* from Tripura forest soil, North-east India

M. Gangotry[1], S.U. Nandhini[1], G. Vijayalashmi[2], K. Manigundan[2], B. Abirami[2] and M. Radhakrishnan[2]*

[1]Department of Biotechnology, Sathyabama Institute of Science and Technology, Chennai-600 119, India

[2]Centre for Drug Discovery and Development, Sathyabama Institute of Science and Technology, Chennai-600 119, India

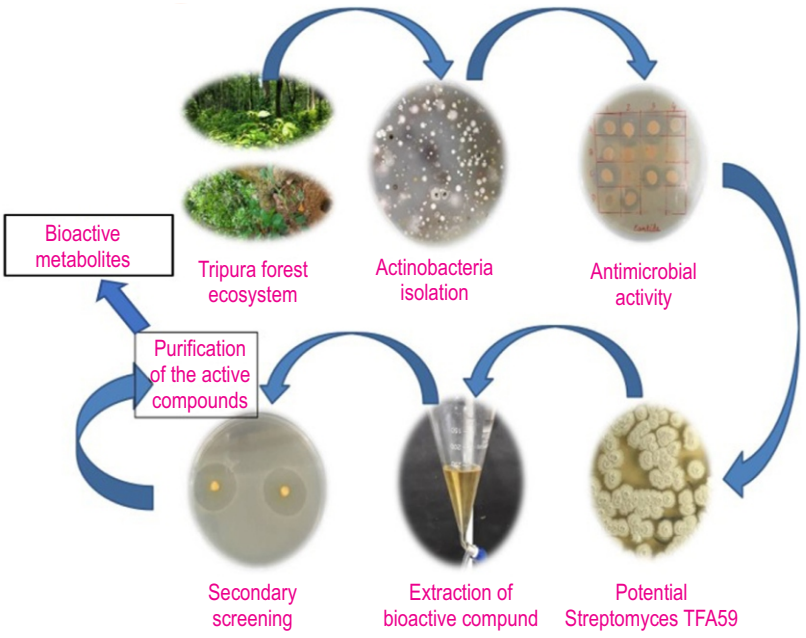
***Corresponding Author Email :** mrkactinos@gmail.com ***ORCiD:** https://orcid.org/0000-0002-2689-6832

Received: 22.10.2020 *Revised*: 23.03.2021 *Accepted*: 02.09.2022

Abstract

Aim: The bioactive potential of Actinobacteria from Tripura forest soil, Northeast India was investigated in this study.

Methodology: Sixty seven actinobacterial isolates recovered from six soil samples were screened for antimicrobial activity against a panel of pathogenic bacteria and fungi. The production of antimicrobial metabolites from the potential strain TFA59 was done by both solid state and submerged fermentation. The antimicrobial compounds were further extracted using ethyl acetate as solvent and the activity was tested against *Staphylococcus aureus* and *Candida albicans*. The actinobacterial strain TFA59 was characterized based on its phenotypic and molecular characteristics.



Results: Antimicrobial activity was exhibited by 28 actinobacterial cultures against atleast one of the test pathogens. Among the solvents tested for extraction, only ethyl acetate extract exhibited activity against *S. aureus* (21 mm) and *C. albicans* (23 mm). The production of antimicrobial compounds by TFA59 was influenced by raffinose, starch, yeast extract and pH 7 when compared with other variables. Based on the phenotypic and molecular characterization, the strain TFA59 was identified as a species of the genus *Streptomyces*.

Interpretation: This study concluded that the *Streptomyces* strains isolated from Tripura forest soil show potential in producing bioactive metabolites that are effective against wide range of pathogens.

Key words: Actinobacteria, Antimicrobial, Bioprospecting, North-east India, *Streptomyces*

How to cite : Gangotry, M., S.U. Nandhini, G. Vijayalashmi, K. Manigundan, B. Abirami and M. Radhakrishnan: Isolation and bioactive potentials of *Streptomyces* from Tripura forest soil, North-east India. *J. Environ. Biol.*, **43**, 764-770 (2022).

Introduction

The prevention and treatment of infectious diseases are at risk due to the rise in the number of microorganisms that might gain resistance to antimicrobial agents (Odonkor and Addo, 2011; Prestinaci *et al.,* 2015). Emergence of drug resistance is the primary cause of worrying rate at which conventional antibiotics are losing their ability to effectively treat infectious microorganisms (Vivas *et al.,* 2019). Only a few numbers of antimicrobial drug alternatives are now available for the treatment of infectious diseases. The pharmaceutical industry has a tremendous challenge in the form of an urgent need for new antimicrobial drugs that are both safe and more effective, especially in view of the growth in antimicrobial resistance (Palanisamy *et al.,* 2017). Many chemicals, including polyenes, are hazardous and thus cannot be used, despite their importance in agriculture, industry, and the treatment of animals (Manyi-Loh *et al.,* 2018). The search for a new antibiotic that is more potent, having a wider spectrum of action and is safer, has so far advanced slowly. Finding new antimicrobial medications that, ideally, arise naturally and have unique mechanisms of action is urgently needed in the medical world.

For instance, soil is a highly exploited ecological niche because the organisms that dwell there produce a large range of advantageous physiologically active natural chemicals, including antibiotics that are crucial to medicine (Atanasov *et al.,* 2021). Among the microbiological sources that live in soil, actinobacteria are the most valuable and well explored. These microorganisms produce physiologically active substances with antibacterial, antifungal, antiparasitic, and anticancer properties (Bhimba *et al.,* 2010; Barka *et al.,* 2016). Fifty percent of all soil actinobacteria are members of the genus *Streptomyces*, and this genus is also the source of seventy-five percent of all commercially and medicinally important antibiotics. Ivermectin, streptomycin, nystatin, and tetracycline are only a few of the antibiotics that are produced by the "Streptomycetes" family of actinobacteria (Pham *et al.,* 2019). Thus, actinobacteria are a contender for the position as a potential solution to these problems. In modern times, the search for actinobacteria in habitats that have previously been investigated often leads to the isolation of well-known actinobacteria as well as antibiotics. Instead, it has been shown that bioprospecting of less investigated geographic regions such as deep forests, caves, marine and deserts are an effective way to find novel bioactive actinobacteria (Radhakrishnan *et al.,* 2014; Sivalingam *et al.,* 2019).

Actinobacteria that produce antibiotics are widely distributed in the forest environment as a result of the ecosystem's distinctive features, which include a great geographic range and a variety of soil types (Aravamuthan *et al.,* 2010; Sharma and Thakur 2020). North-east India's biodiversity has recently attracted a lot of interest. The area contains a wide variety of natural habitats because of its diverse climatic, edaphic, and altitudinal circumstances. It provides a home for a variety of Indian fauna and plants. Contrary to the floral and animal variety, little is known about the microbial diversity in this part of the world. Forests are believed to be the terrestrial ecosystems on our planet with the most diverse plant and animal life (Thakur *et al.,* 2007; Das *et al.,* 2018). They stand as the most comprehensive source from which novel microorganisms and the beneficial natural compounds they create may be obtained. A large number of researchers who were looking for natural compounds with antibacterial activity have found actinobacteria from forest environments in North-east India (Thakur *et al.,* 2007; Das *et al.,* 2018; Sharma and Thakur, 2020). However, the literature reveals that the Tripura State forest in India is still a relatively unknown forest environment for microbial diversity. Thus, this forest ecosystem can be considered as an unexplored source of actinobacteria producing bioactive metabolites. In the current study, soil samples were collected from six different places in Tripura forest area at north-eastern zone of India which maintains the accurate features for the isolation of biologically potential actinobacteria.

Materials and Methods

Isolation of actinobacteria from forest soil: Rhizosphere soil samples were collected from Tripura State forest area (Lat. 24.0672° N; Long. 91.6057° E) located at North-east India and dried at room temperature for 24 hrs. Ten grams of dried sediment sample serially diluted up to 10^{-5} dilutions using sterile distilled water. Hundred microlitre of aliquot from 10^{-3}, 10^{-4} and 10^{-5} dilutions were plated on starch casein nitrate agar (SCA) medium supplemented with nalidixic acid (20 mcg ml^{-1}) and nystatin (50 mcg ml^{-1}). After incubation at 28°C for 1 month, colonies with suspected actinobacterial morphology were recovered, sub-cultured and preserved using YEME agar slants at 4°C. Micromorphology and cultural properties of all the actinobacterial cultures were studied by growing them on YEME agar medium for 7-14 days at 28ºC.

***In-vitro* screening for antimicrobial activity:** Actinobacterial cultures were screened for antimicrobial activity by the agar plug method described by Radhakrishnan *et al.,* (2014). Agar plug with 5 mm diameter of all the actinobacterial cultures were taken and placed over Muller Hinton Agar (MHA) plates inoculated with test bacterial (*Staphylococcus aureus, Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi* and *Pseudomonas aeruginosa*) and fungal (*Candida albicans*) pathogens. The zone of inhibition was measured as millimetre in diameter after 24 hrs of incubation at 37ºC. The actinobacterial strain TFA 59 showed maximum activity and was selected as a potential strain for further studies.

Production and extraction of antimicrobial compounds: Effect of agar surface (solid-state) and submerged fermentation on bioactive compound production by the strain TFA59 was investigated. Spores of the actinobacterial strain TFA 59 were grown on five YEME agar plates and 100 ml of YEME broth. YEME agar plates were incubated at 28°C for 12 days whereas the YEME broth containing flasks were incubated for 12 days in a rotary shaker with 95 rpm. Agar plugs from YEME agar plates was

taken at every 24 hr, and tested against *S. aureus* and *C. albicans*. Each 2 ml of YEME broth was taken and centrifuged at 10000 RPM for 10 min. The cell-free supernatant was tested against *S. aureus* and *C. albicans* by adopting the well diffusion method (Bavya *et al.,* 2011).

Effect of solvents on the extraction of antimicrobial compounds: Antimicrobial compounds secreted by the strain TFA59 on YEME agar medium were extracted by solid- liquid extraction method using n-hexane, chloroform and ethyl acetate. After 24 hr of extraction at room temperature, the solvent portion was separated and concentrated under reduced pressure. Antimicrobial activity of different solvent extracts was tested against *S. aureus* and *C. albicans* by disc diffusion method at 100 mcg per disc concentration (Selvameena *et al.,* 2009).

Effects of medium components on antimicrobial compound production: Effects of different carbon sources, nitrogen sources, minerals and pH on the production of antimicrobial compounds from the strains TFA59 were studied using agar surface fermentation. Strain TFA59 was grown on agar medium supplemented with different carbon, nitrogen and mineral sources and incubated for 10 days at 28°C. The effects of different pH were observed on agar medium adjusted with 0.1 N HCl and 0.1 M NaOH. Agar plugs from all the medium conditions were tested against *S. aureus* and *C. albicans*. Inhibition zone was measured after 24 hr of incubation at 37ºC.

Characterization and taxonomy of potential actinobacterial strain TFA59: Micromorphology of strain TFA59 was studied by slide culture method using YEME agar medium (Balagurunathan *et al.,* 2010). Cultural characteristics were studied using different ISP media (ISP1 – ISP7). The effect of carbon, nitrogen and mineral sources on growth of strain TFA59 was also studied (Rafieenia 2013). The 16S rRNA gene of TFA59 was amplified using the primer pairs: 27F 5´AGAGTTTGATCMTGGCTCAG3´ (forward) and 1492R 5´TACGGYTACCTTGTTACGACTT3´ (reverse) and sequencing was carried out at Eurofins Genomics India Pvt. Ltd., Bangalore. The identification of phylogenetic neighbors and calculation of pair wise 16SrRNA gene sequence similarities were achieved using the MEGA version 7and BLAST analysis (http://blast.ncbi.nlm. nihgov/Blast.cgi). The obtained 16SrRNA sequence was submitted to GenBank to get the accession number. Phylogenetic and molecular evolutionary analyses of potential strain TFA 59 was conducted using software included in MEGA version 7 package.The 16S rRNA sequence of the strains TFA 59 was aligned (http://www.ebi.ac.uk/clustalw) against corresponding nucleotide sequences of representatives of the genus *Streptomyces* retrieved from GenBank. Evolutionary distance matrices were generatedand a phylogenetic tree was

Table 1: Morphological pattern of actinobacterial cultures

Morphological characteristics	Appearance	Number of actinobacterial isolates (%)
Growth	Good	42 (62.68%)
	Moderate	15 (22.38%)
	Poor	10 (14.92%)
Consistency	Powdery	46 (68.65%)
	Sticky	2 (2.98%)
	Leathery	19 (28.35%)
Aerial mass color	White	25 (31.37%)
	Brownish black	3 (4.47%)
	Pale brown	4 (5.97%)
	Grey	10 (14%)
	Yellow	4 (5.97%)
	White	21 (31.34%)
Reverse side pigment	Brown	20 (10.2%)
	Yellow	28 (29.85%)
	Black	7 (10.44%)
Soluble pigment	Brown	8 (11.94%)
	Yellow	5 (7.4%)
Micro-morphology	Aerial and substrate mycelium	67(100%)

Table 2: Effect of solvents on the extraction of bioactive compounds from the strain TFA 59 in both solid and liquid state

TFA 59 extract	Inhibition zone against (mm)	
	S. aureus	*C. albicans*
Ethyl acetate extract	21	23
n-hexane extract	-	-
Chloroform extract	-	-

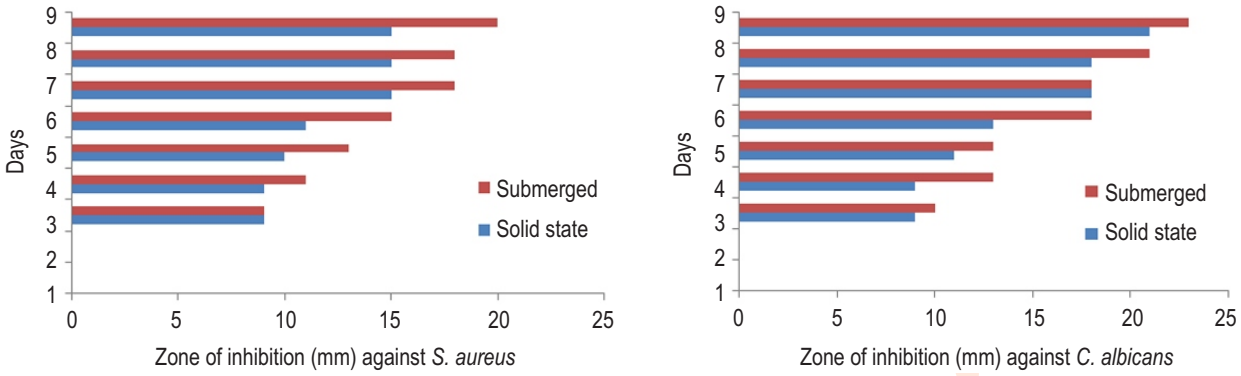


Fig. 1: Effect of fermentation method on bioactive compound production by the strain TFA 59.

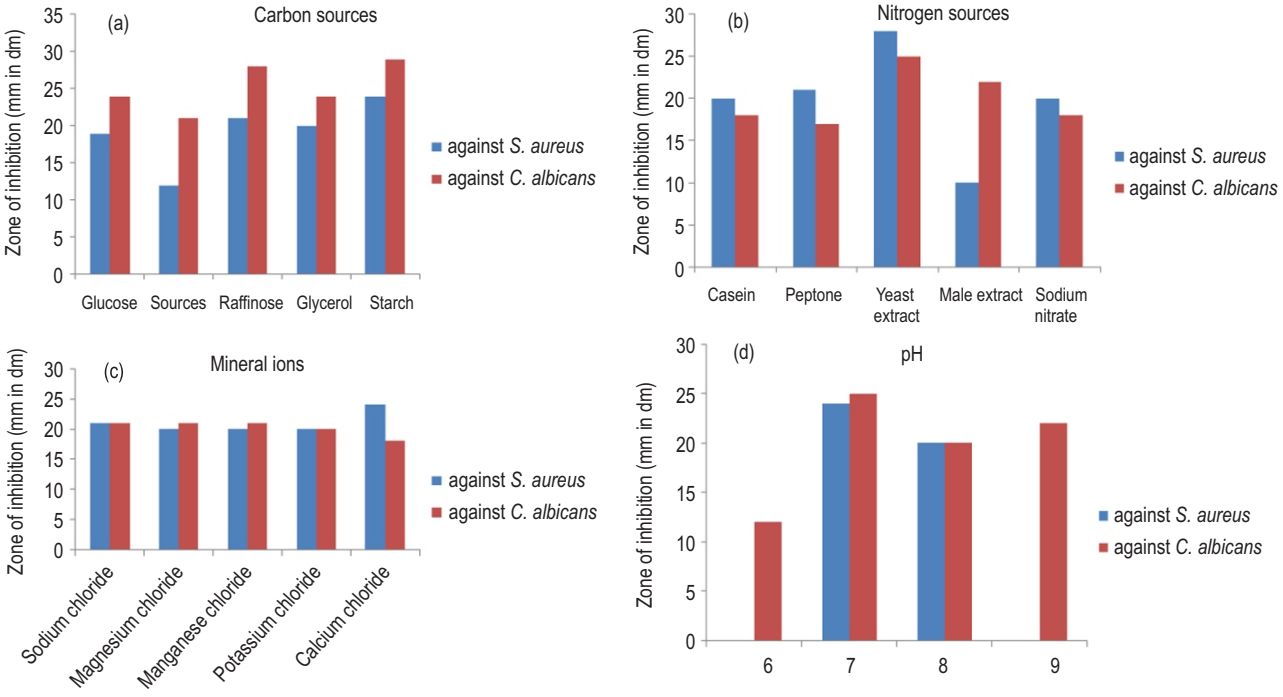


Fig. 2: Effect of medium components on bioactive metabolite production from TFA 59 (a) Carbon sources; (b) Nitrogen sources; (c) Minerals and (d) pH.

inferred by the Neighbor-joining method (Saitou and Nei, 1987). Tree topologies were evaluated by bootstrap analysis based on 1000 resembling of the neighbor-joining data set.

Results and Discussion

North-east India, as a region with diverse climatic and altitudinal variations, is best known for its rich biodiversity. Notably, forests are considered to be the existing promising resource to obtain novel microorganisms and their bio-natural products (Sharma and Thakur, 2020). However, quite a few researchers have reported actinobacteria from the forest ecosystems of North-east India for the search of natural products endowed with antimicrobial activity (Das *et al.,* 2018; Sharma *et al.,* 2016; Thakur *et al.,* 2007). The current study explored the bioactive actinobacteria from the less investigated ecosystems of Tripura forests. Totally, 67 strains (TFA1-TFA67) with actinobacterial morphology were isolated and stored at 4°C in YEME slants for further study. Almost 61% of the isolated cultures displayed good growth on YEME agar medium having varied aerial mass color, reverse side pigments, and few soluble pigments. In a recent study by Sharma and Thakur (2020), out of 107 actinobacterial cultures isolated from the North-eastern state Assam, maximum of 44.8% of the isolates produced white coloured aerial mycelium. In the present study too, a maximum of 31.3% of the isolates produced white colour aerial mycelium. The

Table 3: Characteristics of actinobacterial strain TFA 59

Characteristics		TFA 59
Micromorphology	Aerial mycelium	+
	Substrate mycelium	+
	Fragmentation	-
Cultural characteristics	Colony consistency	Powdery
	Aerial mass colour	Brownish white
	Reverse side pigment	Pale yellow
	Soluble pigment	-
Physiological characteristics	ISP1 (Tryptone yeast extract agar)	+
	ISP2 (Yeast extract malt extract agar)	+
	ISP3 (Oat meal agar)	+
	ISP4 (Inorganic salt starch agar)	+
	ISP5 (Glycerol asparagine agar)	+
	ISP6 (Peptone yeast iron agar)	-
	ISP7 (Tyrosine agar)	+

Fig. 3: Phylogenetic relationship of potential actinobacterial strain TFA59 based on 16S rRNA gene homology. The tree was constructed using the neighbor-joining method with pairwise-deletion model analyses.

pattern of growth and cultural morphology of actinobacterial cultures are tabulated in Table 1. Out of 67 actinobacterial strains, 28 (41.79%) strains were found to show activity against at least one of the six bacterial pathogens tested. Twenty five strains were found to be active against *S. aureus* whereas only four strains (5.9%) inhibited *C. albicans*. No activity was observed against *E. coli* and *K. pneumoniae*. Actinobacteria were screened from diverse habitats for several years for novel bioactive compounds (Lee *et al.,* 2020; Riquelme *et al.,* 2015). However, limited studies have been conducted on the exploration of actinobacteria from forest ecosystem (Bundale *et al.,* 2018; Das *et al.,* 2018; Radhakrishnan *et al.,* 2014). In a previous study Thakur *et al.* (2007), stated that out of nine actinobacterial cultures isolated from Sabahijala Wild life Sanctuary in Tripura state, three isolates showed antimicrobial activity. Only one strain showed inhibition against *S. aureus* and *C. albicans*. Similarly, in this study, only two cultures (TFA 58 and TFA59) showed inhibition against *S. aureus* and *C. albicans*. Notably, the preliminary screening revealed the maximum zone of inhibition by strain TFA59 against the bacterial (*S. aureus*) and fungal pathogen *C. albicans*). Even though, large scale industrial antibiotic production were achieved through submerged process, certain previous studies have reported the production of antibiotics from actinobacteria through solid state fermentation (Bussari *et al.,* 2008; El-Naggar *et al.,* 2009). While certain actinomycetes produce antibiotic compounds in solid medium but not as effectively in liquid medium (Pazhanimurugan *et al.,* 2016), TFA59 produced activity against *S. aureus* and *C. albicans* both in solid state and submerged fermentation even from third day of incubation as shown in Fig. 1.

Majority of the actinobacterial metabolites are extracellular in nature and they are extracted using the medium polar solvent ethyl acetate (Selvameena *et al.,* 2009). Among three different solvents tested for extraction, only ethyl acetate extract showed antimicrobial activity against *S. aureus* and *C. albicans* in both solid state and submerged fermentation, and their zones of inhibition are given in Table 2. Further large scale extraction was done using ethyl acetate. Among the carbon sources, the highest zone of inhibition was observed for starch in both *S. aureus* (24 mm) and *C. albicans* (29 mm). Yeast extract showed 28 mm zone for *S. aureus* and 25 mm zone for *C. albicans*, in nitrogen sources. Calcium chloride was found to influence antimicrobial compound production and the strain TFA59 grown on respective medium was found to produce 24 mm and 28 mm inhibition against *S. aureus* and *C. albicans*, respectively. pH 7 had the maximum effect on antimicrobial compound production from TFA59 with 24 and 25 mm inhibition against *S. aureus* and *C. albicans*, respectively (Fig. 2). Even in previous studies, medium components such as starch, yeast extract and calcium carbonate and pH 7 were found to influence the production of several antibiotics from *Streptomyces* (Rafieenia, 2013). Microscopic observation, showed the presence of aerial mycelium, substrate mycelium, and mycelial fragmentation in TFA59 strain. TFA59 colony appeared powdery with brownish white aerial mass and pale yellow reverse side pigment. No soluble pigment was visible on YEME agar.

Except peptone yeast agar (ISP6), strain TFA59 in remaining agar media (ISP1, ISP2, ISP3, ISP4, ISP5, and ISP7) displayed good growth. Microscopic, cultural and physiological characteristics were shown in Table 3. Molecular strain identification was performed via an analysis of 16S rRNA gene fragment, a beneficial method for identifying micro-organisms up to genus level (Barka *et al.,* 2016). The partial 16S rRNA gene sequence of TFA59 covered a stretch of 1466 bp. It showed 99% similarity with the genus *Streptomyces*. The phylogenetic position of the strain was within a cluster that contains *Streptomyces* sp. 030603 and other species *S. griseus* SYAE50 and *S. albidoflavus* UYFA156 (Fig. 3). The 16S rRNA gene sequence of strain TFA59 was submitted to NCBI-GenBank with the accession number MT351040. Even though there are several antibiotics reported from *Streptomyces*, they still remain as a prolific source for novel bioactive metabolites especially when they are explored from under studied sources like forest ecosystem (Sivalingam *et al.,* 2019). Moreover, it is well known fact that the secondary metabolite production by the actinobacterial members is a strain specific process rather than a species or genus specific process (Sottorff *et al.,* 2019). In addition, the presence of numerous cryptic secondary metabolite biosynthetic gene clusters (smBGCs) in Streptomyces genomes indicates that members of this genus continue to be a valuable source for new drug discovery (Lee *et al.,* 2020). Hence the *Streptomyces* sp. TFA59 explored from the understudied region in Tripura state deserves the potential to isolate antibacterial and antifungal compounds. However, genome based research and isolation of active compounds will pave the way to explore its potential in futhure.

Findings of the present study revealed that the isolation of actinobacteria from underexplored environment serves as a promising source for novel antibiotics.

Acknowledgment

The authors thank the management of Sathyabama Institute of Science and Technology, Chennai for the research facilities provided.

Add-on Information

Authors' contribution: M. Gangotry, G. Vijayalashmi: Laboratory work and data analysis; **K. Manigundan, B. Abirami:** Paper writing and revision; **S.U. Nandhini, M. Radhakrishnan:** Work and guidance.

Research content: The research content of manuscript is original and has not been published elsewhere.

Ethical approval: Not applicable.

Conflict of interest: Authors do not have any conflicts of interest.

Data from other sources: Not applicable.

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology.*

References

Aravamuthan, N., G. Kumar, L. Karthik and K.V. Bhaskara Rao: *In-vitro* antagonistic activity of soil actinobacteria against multi drug resistant bacteria. *Pharmacol. Online*, **2**, 507-516 (2010).

Atanasov, A.G., S.B. Zotchev, V.M. Dirsch and C.T. Supuran: Natural products in drug discovery: Advances and opportunities. *Nature Revi. Drug Disco.,* **20**, 200-216 (2021).

Balagurunathan, R., M. Radhakrishnan and S.T. Somasundaram: L-Glutaminase producing actinomycetes from marine sediments–selective isolation, semi quantitative assay and characterization of potential strain. *Aust. J. Basic. Appl. Sci.,* **4**, 698-705 (2010).

Barka, E. A., P. Vatsa, L. Sanchez, N. Gaveau-Vaillant, C. Jacquard, H.P. Klenk, C. Clément, Y. Ouhdouch and G.P. van Wezel: Taxonomy, physiology, and natural products of *Actinobacteria*. Microbiol. *Mol. Biol. Rev.,* **80**, 1-43 (2016).

Bavya, M., P. Mohanapriya, R. Pazhanimurugan and R. Balagurunathan: Potential bioactive compounds from marine actinomycetes against biofouling bacteria. *Indian J. Geo-Marine Sci.*, **40**, 578-582 (2011).

Bhimba, B.V., J. Meenupriya, E.L. Joel, D.E. Naveena, S. Kumar and M. Thangaraj: Antibacterial activity and characterization of secondary metabolites isolated from mangrove plant *Avicennia officinalis*. *Asian Pac. J. Trop. Med.,* **3**, 544–546 (2010).

Bundale, S., J. Singh, D. Begde, N. Nashikkar and A. Upadhyay: Culturable rare actinomycetes from Indian forest soils: Molecular and physicochemical screening for biosynthetic genes. *Iran. J. Microbiol.,* **10**, 132–142 (2018).

Bussari, B., P.S. Saudagar, N.S. Shaligram, S.A. Survase and R.S. Singhal: Production of cephamycin C by *Streptomyces clavuligerus* NT4 using solid-state fermentation. *J. Ind. Microbiol. Biotech.,* **35**, 49-58 (2008).

Das, R., W. Romi, R. Das, H.K. Sharma and D. Thakur: Antimicrobial potentiality of actinobacteria isolated from two microbiologically unexplored forest ecosystems of Northeast India. *BMC Microbiol.,* **18**, 71 (2018).

Das, R., W. Romi, R. Das, H.K. Sharma and D. Thakur: Antimicrobial potentiality of actinobacteria isolated from two microbiologically unexplored forest ecosystems of Northeast India. *BMC Microbiol.,* **18**, 1-16 (2018).

El-Naggar, M.Y., S.A. El-Assar and S.M. Abdul-Gawad: Solid-state fermentation for the production of meroparamycin by *Streptomyces* sp. strain MAR01. *J. Microbiol. Biotechnol.,* **19**, 468-473 (2009).

Lee, N., W. Kim, S. Hwang, Y. Lee, S. Cho, B. Palsson and B.K. Cho: Thirty complete *Streptomyces* genome sequences for mining novel secondary metabolite biosynthetic gene clusters. *Sci. Data,* **7**, 1-9 (2020).

Manyi-Loh, C., S. Mamphweli, E. Meyer and A. Okoh: Antibiotic use in agriculture and its consequential resistance in environmental sources: Potential public health implications. *Molecules*, **23**, 795 (2018).

Odonkor, S.T. and K.K. Addo: Bacteria resistance to antibiotics: recent trends and challenges. *Int. J. Biol. Med.Res.,* **2**, 1204-1210 (2011).

Palanisamy, S., M. Vinosha, T. Marudhupandi, P. Rajasekar and N.M. Prabhu: *In-vitro* antioxidant and antibacterial activity of sulfated polysaccharides isolated from *Spatoglossum asperum*. *Carbohy. Polym.,* **170**, 296-304 (2017).

Pazhanimurugan, R., M. Radhakrishnan, T. Shanmugasundaram, V. Gopikrishnan, and R. Balagurunathan: Terpenoid bioactive compound from *Streptomyces rochei* (M32): taxonomy, fermentation and biological activities. *World J. Microbiol. Biotechnol.*, **32**, 161 (2016).

Pham, J.V., M.A. Yilma, A. Feliz, M.T. Majid, N. Maffetone, J.R. Walker and Y.J. Yoon: A review of the microbial production of bioactive natural products and biologics. *Front. Microbiol.,* **10**, 1404 (2019).

Prestinaci, F., P. Pezzotti and A. Pantosti: Antimicrobial resistance: A global multifaceted phenomenon. *Pathog. Glob. Hlth.,* **109**, 309-318 (2015).

Radhakrishnan, M., V. Gopikrishnan, S. Balaji, R. Balagurunathan and V. Kumar: Bioprospecting of actinomycetes from certain less explored ecosystems active against *Mycobacterium tuberculosis* and other non-mycobacterial pathogens. *Int. Sch. Res. Noti.*, **2014**, 812974 (2014).

Rafieenia, R.: Effect of nutrients and culture conditions on antibiotic synthesis in *Streptomycetes*. *Asian J. Pharm. Hlth. Sci.,* **3,** 810-815 (2013).

Riquelme, C., J.J. Marshall Hathaway, M.D.L. Enes Dapkevicius, A.Z. Miller, A. Kooser, D.E. Northup, J. Valme, F. Octavio, S. Cesareo and N. Cheeptham: Actinobacterial diversity in volcanic caves and associated geomicrobiological interactions. *Front. Microbiol.,* **6**, 1342 (2015).

Saitou, N. and M. Nei: The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.,* **4**, 406-425 (1987).

Selvameenal, L., M. Radhakrishnan and R. Balagurunathan: Antibiotic pigment from desert soil actinomycetes; biological activity, purification and chemical screening. *Indian J. Pharm. Sci.,* **71**, 499 (2009).

Sharma, P. and D. Thakur: Antimicrobial biosynthetic potential and diversity of culturable soil actinobacteria from forest ecosystems of Northeast India. *Sci. Rep.,* **10**, 1-18 (2020).

Sharma, P., M.C. Kalita and D. Thakur: Broad spectrum antimicrobial activity of forest-derived soil actinomycete, *Nocardia* sp. PB-52. *Front. Microbiol.,* **7**, 347 (2016).

Sivalingam, P., K. Hong, J. Pote and K. Prabakar: Extreme environment *Streptomyces*: Potential sources for new antibacterial and anticancer drug leads?. *Int. J. Microbiol.,* **2019**, 5283948 (2019).

Sottorff, I., J. Wiese, M. Lipfert, N. Preußke, F.D. Sönnichsen and J.F. Imhoff: Different secondary metabolite profiles of phylogenetically almost identical *Streptomyces griseus* strains originating from geographically remote locations. *Microorganisms*, **7**, 166 (2019).

Thakur, D., A. Yadav, B.K. Gogoi and T.C. Bora: Isolation and screening of *Streptomyces* in soil of protected forest areas from the states of Assam and Tripura, India, for antimicrobial metabolites. *J. Mycol. Médi.,* **17**, 242-249 (2007).

Vivas, R., A.A.T Barbosa, S.S. Dolabela and S. Jain: Multidrug-resistant bacteria and alternative methods to control them: An overview. *Microb. Drug. Resist.,* **25**, 890-908 (2019).