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Research Article

SCREENING OF ACTINOMYCETES ISOLATED FROM SOIL SAMPLES FOR ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY

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ABSTRACT

Objective: screening the antibacterial and antioxidant activity of actinomycetes isolated from three different garden soil samples of VIT University, Vellore, Tamil Nadu,India.

Methods: All the isolates were evaluated for their inhibitory activities against four clinical pathogens by agar well diffusion method and assessed for its antioxidant potential using DPPH scavenging assay. The presence of volatile compounds in crude extract was analyzed by Gas chromatography–mass spectrometry (GC-MS) analysis.

Results: It was observed that one strain AC 23 showed broad spectrum activity at the concentration of 30mg/ml against *Bacillus cereus* (35mm), *Staphylococcus aureus* (38mm), *Pseudomonas aeruginosa* (19mm), *Escherichia coli* (40mm), *Salmonella typhi* (22mm). The antioxidant potential of the crude extract was found maximum at the concentration of 20mg/ml with 60% inhibition. Further the cultural characteristics of the strain AC23 was studied on different culture media. The 16srRNA gene sequence of the strain AC23 revealed the degree of sequence similarity 98% with *Streptomyces werraensis* and it was validated by existing taxonomic reports.

Conclusion: These findings may have capability to identify some of the most important lead compounds ever developed.

Keywords: Soil actinomycetes, Antibacterial activity, Antioxidant activity, Bioactive compounds.

INTRODUCTION

Many soil-inhabiting bacteria are known to produce secondary metabolites that can suppress microorganisms competing for the same resources [1]. Microbial population play's a prominent role for biotechnology and pharmaceutical industries as it offers countless new genes and biochemical pathways to probe for enzymes, antibiotics and other useful molecules. There are a number of microorganisms which produce a number of medically and industrially useful compounds which is primarily bioactive secondary metabolites. Actinomycetes are diverse group of gram positive bacteria that usually grow by filament formation. Actinomycetes are characterized by a complex life cycle and they belong to the phylum Actinobacteria that represents one of the largest taxonomic units among the 18 major lineages currently recognized within the domain bacteria [2]. They have high G+C (>55%) content in their DNA. Around 23,000 bioactive secondary metabolites produced by microorganisms have been reported and over 10,000 of these compounds are produced by actinomycetes, representing 45% of all bioactive microbial metabolites discovered. Actinomycetes are the most economically and biotechnologically valuable prokaryotes and are responsible for the production of about half of the discovered bioactive secondary metabolites, antibiotics, anticancer agents and enzymes [3]. Among actinomycetes, around 7,600 compounds are produced by *Streptomyces* species [4]. They are the best common source of antibiotics, and provide approximately two-third of naturally occurring antibiotics, including many of medical importance discovery of antibiotics from microbial sources have yielded an impressive number of compounds over the past 50 years [5]. The present scenario demands the discovery of new antimicrobial agents which can be of a better potential and activity than the previously discovered agents. The reason for this demand is the emergence of the new kind and common pathogens which are multidrug resistant. Resistance of bacteria to the effects of antibiotics has been a major problem in the treatment of diseases. Infectious diseases are still the second leading cause of death worldwide [6]. To solve the problem of these multidrug resistance pathogens more sources of antimicrobial agents need to discovered and studied. The present study is focused on bioactive properties of actinomycetes from garden soil sample which are known to be a promising source of bioactive metabolites.

MATERIALS AND METHODS

Sample collection

A total of four garden soil samples were collected randomly from different location of VIT University, Vellore, Tamil Nadu, India. The depth of 5 cm below the surface of the soil were chosed and the soil samples were brought to the laboratory in sterile polythene bags and refrigerated

Isolation of actinomycetes

Twenty four actinomycetes strain were isolated and obtained as pure culture by using standard microbiological method. From each soil sample, 1 gm of dried soil was suspended in 9 mL sterile water, and successive serial dilutions were made by transferring 1mL of aliquots to 2nd test tube containing 9 mL of sterile water, and in this way dilutions up to 10^{-4} were prepared. Each time the contents were vortexed to form uniform suspension. An aliquot of 0.1 mL of each dilution was taken and spread evenly over the surface of starch casein agar medium supplemented with cycloheximide (100µg/mL). Plates were incubated at 27°C and monitored for 7 days. Colonies those showed *Streptomyces* like appearance under light microscope were recultivated several times for purity. The purified actinomycetes were preserved on starch casein agar slants at 4°C for two months and at −20°C in the presence of glycerol (15%v/v) for longer periods.

Color grouping of the isolates

The color of the aerial mycelia & pigment production by the isolates were determined on starch casein agar plates after 7 days of incubation at room temperature. The color of the substrate mycelia and those of the soluble pigment were determined according to National Bureau of Standard Color Chart [7].

Preliminary screening - cross-streak method

Isolated strains AC1 to AC24 were inoculated onto modified nutrient agar plates by single streak on the center. The plates were incubated at 30 °C for 3 days. Five bacterial pathogens *namely Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli, Salmonella typhi* were streaked perpendicular to the antagonist on the agar medium. The plates were incubated at 37 °C for 24 h. The microbial inhibitions were observed by determining the diameter of the inhibition zones [8]

Extraction of antimicrobial compounds

The selected antagonistic isolate AC23 were inoculated into starch casein broth and incubated at 30 °C in a shaker at 200 rpm for seven days. After incubation the broth was collected and filtered through what man No. 1 filter paper. Further the filtrates were centrifuged at 5 000 rpm for 10 min. The supernatant was transferred aseptically into a screw capped bottles and stored at 4 °C for further assay [9].

Secondary screening-Agar-well diffusion method

The antibacterial activity of the isolate *AC23* crude ethyl acetate extract was analyzed by agar well diffusion method [10]. All the pathogenic inoculums namely *Salmonella typhi* (MTCC No: 1167), *Bacillus cereus* (MTCC No: 6840), *Staphylococcus aureus* (MTCC No: 7405), *Pseudomonas aeruginosa* (MTCC No: 4676), *Escherichia coli (*MTCC No: 1588) were swabbed onto the surface of Mueller-Hinton agar plates and was dung using sterile cork borer (diameter = 5mm). The extract was sterilized through a 0.22 m filter and was impregnated with 100 µL. Chloramphenicol 25ug/ml was used as positive control. All plates were incubated at 37°C for 24 hrs. The diameter of the minimum zone of inhibition was measured in mm.

Free radical Scavenging Activity

The antioxidant activity of AC23 crude ethyl acetate extract was determined by DPPH scavenging assay [11]. Various concentration (0.1, 0.5, 1.0 & 5.0 mg/ml) of crude extract were taken in separate tubes. Ascorbic acid was used as reference compound (0.2, 0.4, 0.8.1.0 & 5.0 mg/ml). A freshly prepared solution of 0.002% DPPH (1, 1, Diphenyl-2-Picryl hydrazyl) was freshly prepared in methanol. DPPH (2 ml) was added to each tube containing different concentrations of extract (2 ml) and of standard solution (2 ml). The samples were incubated in dark place at 37 °C for 20 min and read at 515 nm containing 100 µL of methanol in the DPPH solution was prepared as blank sample and its absorbance was measured (*A*B) all the experiment was carried out in triplicate. The data were expressed as the percent decrease in the absorbance compared to the control. Percentage inhibition of radical scavenging activity was calculated using formula

Percent DPPH scavenging activity = Absorbance (control)-Absorbance (Test) x 100

Absorbance (control

Taxonomic investigation of the potent stain

The isolate AC23 were phenotypically grouped according to the traditional criteria of classification including growth, aerial spore-mass color, texture, elevation, substrate mycelium color, margin, pigment production. The species was identified based on methods followed [12, 13, 14].

Molecular charecterisation

Extraction of genomic DNA

The potent actinomycete isolate AC23 was cultured in SC broth and incubated at 28°C for 7 days in an orbital shaker at 130 rpm. Genomic DNA was extracted using Fungal Genomic DNA Isolation Kit RKT 41/42 (chromous Biotech Pvt. Ltd., Bangalore, India) according to the manufacturer instructions and visualized using 0.8% (w/v) agarose gel electrophoresis.

PCR

The PCR amplification reactions were performed in a total volume of 25 µL. Each reaction mixture contained the following solutions: 1 µL genomic DNA, 1 µL 10 pmol forward 16S rDNA primer 27F (5'-AGAGTTTGATCMTGGCTCAG -3'); 1 µL of 10 pmol reverse 16S rDNA primer 1492R (5'- TACGGYTACCTTGTTACGACTT -3'); 1 µL of 30 mM deoxyribonucleoside 5'-triphosphate (N= A,T,G,C) (dNTP's); 2.5 µL of 10X PCR buffer and 1 µL Taw polymerase (1 U) and water was added up to 25 µL. The thermal cycler (MJ Research PTC 200, USA) was programmed as follows: 2 min initial denaturation at 94°C, followed by 30 cycles that consisted of denaturation for 45 sec at 94°C, annealing for 60 s at 55°C and extension at 72°C for 60sec and a final extension of 5 min at 74°C. The PCR amplified product was analyzed by 1.2% agarose gel electrophoresis using TAE buffer. The resulting DNA patterns were examined with UV light under transilluminator, photographed and analyzed using gel documentation system (Hero labs, Germany).

16S r DNA sequencing

The 16S rDNA of potent isolate AC23 were sequenced by using Big Dye terminator cycle sequencing kit (Applied Bio Systems, USA). The Sequenced products were resolved on an Applied Bio systems model 3730XL automated DNA sequencing system (Applied Bio Systems, USA). The sequences were subjected to homology search using BLAST programme of the National Center for Biotechnology Information (NCBI). [15]. A phylogenetic tree was constructed by neighbor- joining method with the programme Tree view (Version 1.6.6). Individual branches in phylogenetic tree were determined by bootstrap analysis based on 1000 samplings.

RNA Structure Prediction

The gene sequence was transcribed to RNA sequence to identify the rRNA secondary structure using the RNA fold server from Vienna RNA website. RNA secondary structure can be predicted by free energy minimization with nearest neighbor parameters to evaluate stability. It was used to predict the minimum free energy (MFE) secondary structure of single sequences using the dynamic programming algorithm proposed and equilibrium base-pairing probabilities are calculated using partition function (PF) algorithm. [16, 17]

GC-MS analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25 mm ID ×1EM df, composed of 100% Dimethyl poly siloxane, operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 EI was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C/min, then 5°C/min to 280°C/min, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da. Interpretation on mass spectrum of GC-MS was done using the database NIST08 and WILEY8.The relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

RESULTS

A total of four garden soil samples were collected from different locations of VIT University Vellore, Tamil Nadu. Twenty four organisms were isolated based on the morphology (Table 1,2).

Table 1: Soil samples collected from different locations of VIT University, Vellore.

S. No.	Sampling spots	Samples	Number of isolated
1	Garden	VIT University garden Soil	6
2	Garden	VIT University garden Soil	4
3	Garden	VIT University garden Soil	5
4	Garden	VIT University garden Soil	9

Table 2: Morphological characterization of Actinomycete isolates

S. No.	Isolate Name	Aerial Mycelium	Substrate Mycelium	Pigment Color	Antioxidant Activity	Antibacterial Activity
1	AC-1	Light grey	Grey	-	-	-
2	AC-2	Dark grey	Grey	-	-	-
3	AC-3	Dark grey	Grey	-	-	-
4	AC-4	Light grey	Light grey	-	-	-
5	AC-5	Whitish grey	Grey	-	-	-
6	AC-6	Milky white	Milky white	-	-	-
7	AC-7	Dark grey	Dark grey	-	-	-
8	AC-8	White	White	Light yellow	-	-
9	AC-9	White	Grey	-	-	-
10	AC-10	White	Light grey	-	-	-
11	AC-11	Light grey	Dark grey	-	-	-
12	AC-12	Bright white	White	Yellow	-	-
13	AC-13	Dark green	Dark green	-	-	-
14	AC-14	Light grey	White	Yellow	-	-
15	AC-15	Light grey	Grey	-	-	-
16	AC-16	Light orange	White	Yellow	-	-
17	AC-17	White	Dark green	-	-	-
18	AC-18	Light grey	White	-	-	-
19	AC-19	White	Dark green	-	-	-
20	AC-20	Bright white	Milky white	Light yellow	-	-
21	AC-21	Light grey	Dark grey	-	-	-
22	AC-22	Grey	Yellowish white	-	-	-
23	AC-23	White	Grey	Yellow	+	+
24	AC-24	Green dark	Light grey	Yellow	-	-

All the isolates were subjected to preliminary screening by cross streak assay. Out of which one strain AC3 exhibited potent activity. (Figure. 1)

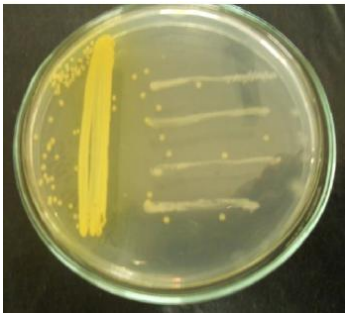


Fig. 1: Cross streak assay of the strain AC23

The bioactive potential of the strain AC23 was further studied on the basis of its morphological and chemical properties. The morphological characterization of the strain was done based on the, color of aerial and substrate mycelia, shape, ornamentation of spore surface.(Table 3,4,5).

Table 3: Morphological properties of strain AC 23

Morphological properties						
Sporophore morphology	Spore surface	Colour of aerial mycelium	Colour of substrate mycelium	Gram staining	Acid fast staining	Motility
Spiral	Smooth	White	Grey	+	Non-acid fast	-

Table 4: Biochemical Properties of strain AC 23

Morphological properties						
Sporophore morphology	Spore surface	Colour of aerial mycelium	Colour of substrate mycelium	Gram staining	Acid fast staining	Motility
Spiral	Smooth	White	Grey	+	Non-acid fast	-

Table 5: Cultural properties of strain AC 23

Medium	**Growth**
ISP medium 1	Good
ISP medium 2	Very good
ISP medium 3	Good
ISP medium 4	Good
ISP medium 5	Good
ISP medium 6	Better
ISP medium 7	Good
ISP medium 8	Good
ISP medium 9	Good
Bennett agar	Good
Nutrient agar	Better
Czapex Dox agar	Good
Kenknight's agar	Very good
Actinomycete isolation agar	Very good
Starch casein agar	Very good

The taxonomic studies of the strain AC23 revealed that it belongs to the genera *Streptomyces* sp. The microscopy of strain AC23were examined and presented in (Figure. 2).

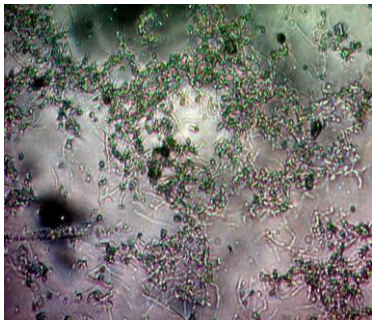


Fig 2: Microscopy of strain AC23

The Biochemical and cultural Properties of the strain VITJS3 was characterized. The effect of pH, Temperature, NaCl, Carbon and Nitrogen sources were optimized (Table 6, 7, 8, 9,10).

Table 6: Effect of temperature on strain AC 23

Effect of Temperature				
15°C	28 °C	37 °C	45 °C	50 °C
-	+	+	-	-

Table 7: Effect of pH on strain AC 23

Effect of pH				
5	6	7	7.5	8
-	-	+	+	-

Table 8: Effect of NACL tolerance on strain AC 23

NACL tolerance(%W/V)				
0.5	1	2	3	4
+	-	-	-	-

Table 9: Effect of carbon source on strain AC 23

Carbon source (1% w/v)							
D-glucose	Sucrose	D-galactose	Mannose	Maltose	Lactose	Mannitol	L-Rhamnose
+	-	-	+	-	-	+	-

Table 10: Effect of nitrogen source on strain AC 23

Nitrogen Sources(1%w/v)								
Cysteine	Arginine	Threonine	Alanine	Aspartic acid	Glycine	Histidine	Lysine	Phenyl alanine
-	+	-	-	-	-	+	-	+

The bioactive compounds were extracted from the fermentation broth using ethyl acetate solvent and which was subsequently evaporated to obtain the crude extract. The antibacterial activity of ethyl acetate crude extracts at the concentration of 20mg/well was assayed by agar well diffusion method against pathogens *Bacillus cereus* (35mm), *Staphylococcus aureus* (38mm), *Pseudomonas aeruginosa* (19mm), *Escherichia coli* (40mm), *Salmonella typhi* (22mm). **(**Figure 3).

1 2 3 4 5

Fig. 3: Antibacterial activity of strain AC23 against pathogens

1: *Pseudomonas aeruginosa* MTCC No: 4676 (19mm), 2: *Staphylococcus aureus* MTCC No: 7405(38mm), 3: *Escherichia coli* MTCC No: 1588(40mm), 4: *Salmonella typhi* MTCC No: 1167 (21mm), 5: *Bacillus cereus* MTCC No: 6840(35mm),

The antioxidant activity of the VITJS3 ethyl acetate crude extract showed highest activity at 5µg/ml with 60 % inhibition; however its activity is comparable and remained highly significant with the reference used. (Figure 4).

The 16S rRNA gene sequence analysis was performed to confirm the identification of isolate AC23, The sequence alignment using BLASTN software for the comparison gave high homology (99%) with *Streptomyces werraensis.* A phylogenetic relationship was established through the alignment and cladistic analysis.(Figure 5).

The partial 16S rRNA sequences derived in this study were deposited in Gen Bank under the accession number KC961639.The secondary structure was predicted using RNA fold server (using MFE) with a minimum free energy of -398.26 kcal/mol. Based on the energy of the predicted models, the secondary structure predicted by MFE gave the more stable structure with a minimum energy. Hence MFE model can be considered as the optimal secondary structure of the rRNA. Mountain plot was also derived from RNA fold server which represents a secondary structure in a plot of height versus position, where the height is given by the number of base pairs enclosing the base at position i.e. loops correspond to plateaus (hairpin loops are peaks), helices to slopes (Figure 6)

Identification of the volatile components was carried out using GC-MS analysis. The GC-MS chromatogram of the AS3 crude extract showed 3 major peaks indicating the presence of bioactive constituents. Hence several important organic metabolites which includes BENZOIC ACID, 4-ETHOXY-, ETHYL ESTER, TRANS-2,4-DIMETHYLTHIANE,S,S-DIOXIDE,TRANS-2-METHYL-4-N-PENTYLTHIANE, S,S-DIOXIDE (Figure 7, Figure 8)

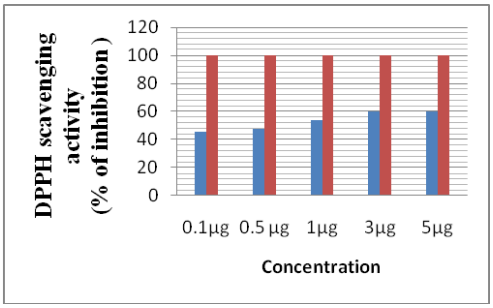


Fig 4: Comparable antioxidant activity of strain AC23 crude extract with standard ascorbic acid

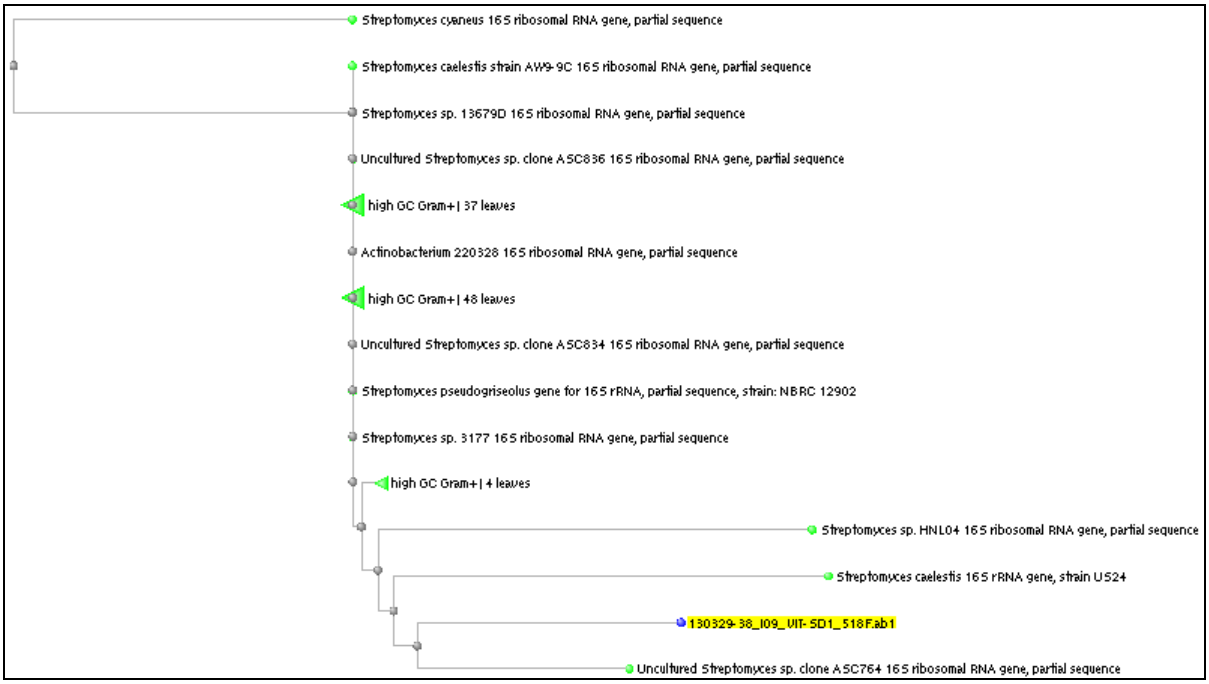
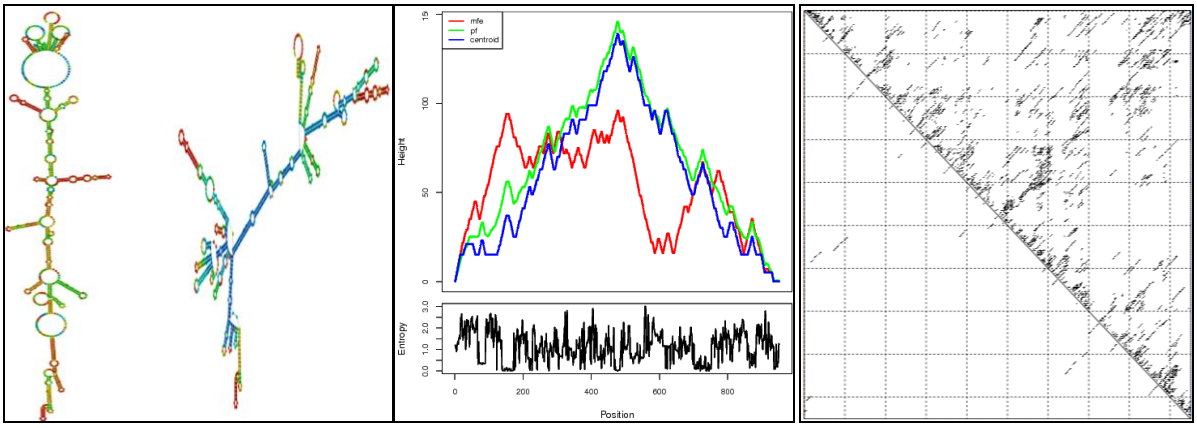


Fig. 5: Phylogenetic tree of strain AC23



(A) (B) (C)

Fig. 6: RNA Secondary structure (A) RNA secondary structure, (B) Mountain plot representation (C) Base pair probabilities of Dot plot

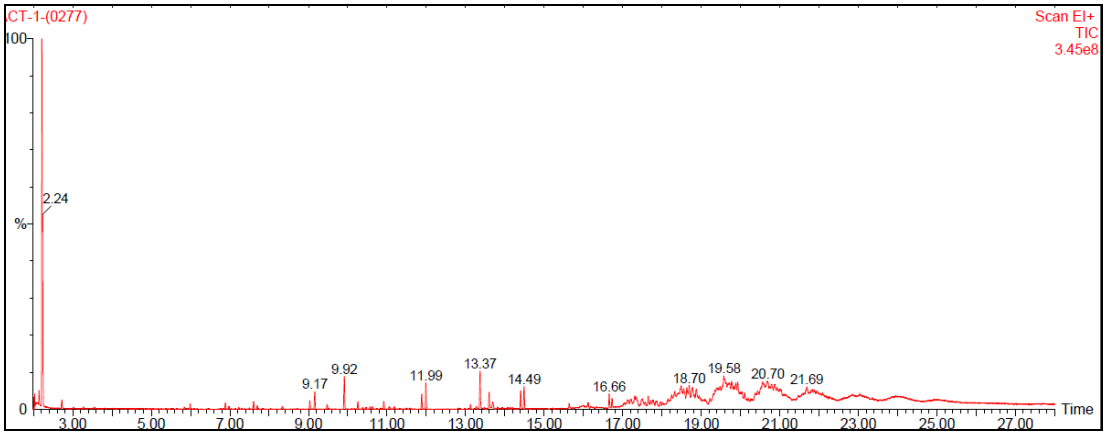
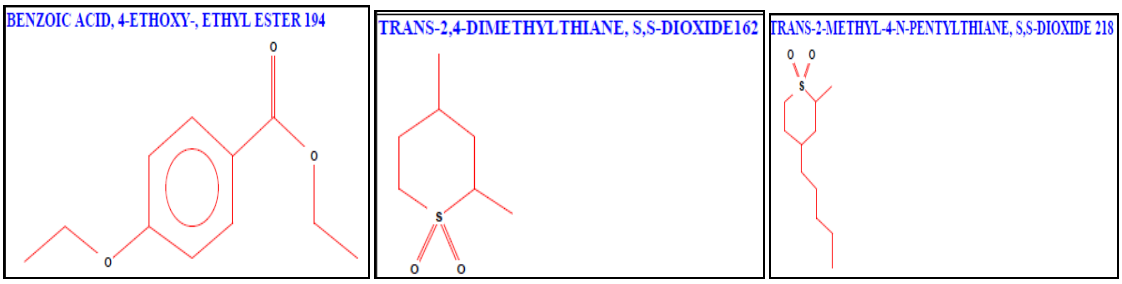


Fig. 7: The GC-MS chromatogram of the strain AC23 crude ethyl acetate extract



(A) (B) (C)

Fig. 8: Molecular structure and corresponding GC-MS peaks of major bioactive metabolites from crude shell extract of AC 23

(A) BENZOIC ACID, 4-ETHOXY-, ETHYL ESTER, (B) TRANS-2,4-DIMETHYLTHIANE, S,S-DIOXIDE, (C) TRANS-2-METHYL-4-N-PENTYLTHIANE, S,S-DIOXIDE .

Interpretation on mass spectrum of GC-MS was done using the database NIST08, WILEY8. Hence the presence of various bioactive compounds justifies the use of the crude shell extract for various ailments of diseases.

DISCUSSION

The need for the discovery of effective new antimicrobial compounds is very important. The approach to new drugs through natural products has proved to be the single most successful strategy for the discovery of new drugs[18]. The discovery of new active metabolites must be followed by adequate biological testing [19] which will require the immediate availability of substantial amounts of naturally derived material. One way to prevent antibiotic resistance is by using new compounds which are not based on existing synthetic antimicrobial compounds. Searching for new microbial metabolites requires strategies directed at decreasing the probability of identifying known compounds. This study was performed with an aim of isolating actinomycetes strains with antimicrobial activities using the selective isolation media and with an objective of identifying culturable new actinomycetes. The present study indicated existence of a diverse group of actinomycetes in the soil which resulted in selective isolation of soil *Streptomyces* with antibacterial and antioxidant activity. GC-MS analysis of crude extract of *Streptomyces werraensis* clearly demonstrated the presence of several important organic metabolites which are known antagonist to bacteria, viruses, and fungi [20, 21, 22, 23, 24]. A total of 110 actinomycetes strains were isolated from the soil samples collected from the protected forest soil from two states in Northeast India. These were then characterized by conventional methods and assessed for their antagonistic activity preliminary against test microorganisms [25]. Previous reports have shown in vitro antimicrobial activity of *Streptomyces* spp. ERI-3 isolated from Western Ghats rock soil [26]. The isolation of actinomycetes from the soils with maximum antibacterial activity against *Pseudomonas aeruginosa* (20mm) and antioxidant activity at the maximum inhibition at 50mg/ml concentration with 90.7% [27]. Actinobacteria not only maintain the pristine nature of the environment, but also serve as biological mediators through their involvement in biogeochemical processes [28]. Hence this work highlight the importance and indicates that the actinomycetes are the major useful source of novel class of lead for many applications such as control of infectious diseases and drug discovery. But more precise work and further development in this field is required to produce more potent bioactive compounds from actinomycetes which are easily available in the soil.

CONCLUSION

Comparing the above mentioned results with this study, we can conclude that the garden soil samples collected from different locations represents the rich source of actinomycetes. It is confirmed from the present observation, actinomycetes in the soil resides the potential sources of unique natural bioactive metabolites. Future studies will be focused as to identify the active lead molecule.

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REFERENCES

1. Paolina Garbeva, Olaf Tyc, Mitja NP, Remus-Emsermann, Annemieke van der Wal, Michiel Vos, Mark Silby, Wietse de Boer No apparent costs for facultative antibiotic production by the soil bacterium *Pseudomonas fluorescens* Pf0-1. PLOS ONE. 2011; 6: e27266.
2. Usha R, Anathasevi P, Venil CK, Palaniswamy Antimicrobial and Antiangiogenesis Activity of *Streptomyces paravulus* KUAP106 from Mangrove Soil. European Journal of Biological Sciences. 2012; 4(4): 77-83.

3. Kumari Amrita, Jain Nitin, Subathra Devi C Novel bioactive compounds from mangrove derived actinomycetes. International research journal of pharmacy. 2012; 3(9): 26-29.
4. Olano C, Carmen Méndez, José A, Salas Antitumor Compounds from Marine Actinomycetes. Marine Drug. 2009; 7(2): 210-248.
5. Okami Y, Hotta K. Search and discovery of new antibiotics. In: Good fellow M, Williams ST, Mordarski M, editors. Actinomycetes in Biotechnology. London, UK,Academic press ;1988. p. 37–67.
6. Luzhetskyy A, Pelzer S, Bechthold A The future of natural products as a source of new antibiotics. Current opinion in investigational drugs. 2007; 8 (8): 608-613.
7. Zhao H, Parry RL, Ellis DI, Griffith GW, Good acre R The rapid differentiation of Streptomyces isolates using fourier transform infrared spectroscopy. Vibrational Spectroscopy. 2006; 40(2): 213–218.
8. Lemons ML, Toranzo AE, Barja JL Antibiotic activity of epiphytic bacteria isolated from intertidal seaweeds. Microbial Ecology. 1985; 11:149–163.
9. Sambamurthy R, Ellaiah P A new *Streptomycin* producing neomycin (B and C) complex, *Streptomyces marinensis* (part-1) Hind Antibiot Bull. 1974; 17:24–28.
10. Omar S, Lemonnier B, Jones N, Ficker C, Smith ML, Neema C, Towers GHN, Goelk, ArnasonJTAntimicrobial activity of extracts of eastern North American hardwood trees and relatio n to traditional medicines. Journal of Ethnopharmacology. 2000*;* 73:161-170.
11. Miliauskas G, Venskutonis PR, Van Beek TA Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chemistry. 2004; 85:231-237.
12. Shirling EB, Gottlieb D Methods for characterization of *Streptomyces* species. International Journal of Systematic Bacteriology. 1966; 16: 313–340
13. Nonomura H. Key for classification and identification of 458 species of the Streptomycetes included in ISP. Journal of Fermentation Technology. 1974; 52(2):78–92
14. Buchanan RE, Gibbons NE Bergey's manual of determinative bacteriology, 8th edn. The Williams and Wilkins Co., Baltimore: 1974; 1146
15. Gomaa EZ. Chitinase Production by *Bacillus thuringiensis* and *Bacillus licheniformis*: their Potential in Antifungal Bio control. Journal of Microbiology. 2012; 50(1):103–111
16. Zuker M, Stiegler P Optimal computer folding of large RNA sequences using thermodynamics and auxiliary information. Nucleic Acids Research. 1981; 9: 133-148.
17. McCaskill JS. The equilibrium partition function and base pair binding probabilities for RNA secondary structure. Biopolymers. 1990; 29: 1105-1119.
18. Soumya Prakash Rout, Choudary KA, Kar DM, Lopamudra Das, Avijeet Jain, Plants in traditional medicinal system - future source of new drugs. International Journal of Pharmacy and Pharmaceutical Sciences. 2009 1, 1:1-23.
19. Jemimah Naine S, Mohana srinivasan V, Subathra Devi C Novel anticancer compounds from marine actinomycetes. Journal of Pharmacy Research. 2011; 4 (4): 1285-1287.
20. Rahman H, Austin B, Mitchell WJ, Morris PC, Jamieson AJ, Adams DR, Spragg AM, Schweizer M Novel anti infective compounds from marine bacteria. Marine Drugs. 2010; 8: 498–518.
21. Burkholder R, Pfister M, Leitz FH Production of a pyrrole antibiotic by a marine bacterium. Applied Microbiology. 1966; 14(4): 649–653
22. Snipes S, Person A, Keith, Cupp J Butylated hydroxytoluene inactivates lipid containing viruses. Science. 1975; 188(4183): 64–66.
23. Proestos IS, Boziaris M, Kapsokefalou, Komaitis M. Natural antioxidant constituents from selected aromatic plants and their antimicrobial activity against selected pathogenic microorganisms. Food Technology and Biotechnology. 2008; 46(2):151–156.
24. Mishra Sree A. Comparison of the antibacterial activity, volatiles and fatty acid composition of lipids of *Phycopsis* species collected at different locations from the Bay of Bengal (Orissa coast). Journal of the Serbian Chemical Society. 2009; 74(2): 133–139.
25. Thakur D,Yadav A, Gogoi BK, Bora TC Isolation and screening of *Streptomyces* in soil of protected forest areas from the states of Assam and Tripura, India, for antimicrobial metabolites. Journal of Medical Mycology. 2007; 17:242- 9.
26. Valan Arasu M, Duraipandiyan V, Agastian P, Ignacimuthu S In vitro antimicrobial activity of *Streptomyces* spp. ERI-3 isolated from Western ghats rock soil (India). Journal of Medical Mycology. 2009;19: 22- 8.
27. Jemimah Naine S, Nasimunislam N, Vaishnavi B, Mohanasrinivasan V, Subathra Devi C Isolation of soil actinomycetes inhabiting amrithi forest for the potential source of bioactive compounds. Asian Journal of Pharmaceutical and Clinical Research. 2012; 5(4): 189-192.
28. Karthik L, Gaurav kumar K, Bhaskara rao V Diversity of marine actinomycetes from nicobar marine sediments and its antifungal activity, International Journal of Pharmacy and Pharmaceutical Sciences. 2010: 2(1):199-203.