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Screening and characterization of actinomycetes with antibacterial activity from shore line soil of Tiruchendur

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ABSTRACT

The aim of the present study was to screen, characterize and to identify the actinomycetes having antibacterial activity. The actinomycetes isolates obtained from coastal soil of Tiruchendur Tamil Nadu, India were screened for their antibacterial activity by Kirby Bauer well diffusion method. In screening, three actinomycetes Tir7, Tir12 and Tir13 were selected for further studies. Morphological, Biochemical and Physiological characteristics were studied to characterize the actinomycetes. The three isolates were mass cultured in ISP2 broth and the bioactive metabolites were isolated using centrifugation phase separation method by adding equal amount of eight different solvents. Bacterial strains such as *Staphylococcus aureus, Micrococcus luteus, Bacillus cereus, Proteus mirabilis, Proteus vulgaris, Neisseria mucosa,* and *Enterococcus faecalis* were obtained from MTCC, Chandigarh, were challenged with the extracts by Kirby Bauer well diffusion method. Isolate Tir12 was selected as the most potent actinomycete among the three isolates and the solvent ethyl acetate and chloroform extracted the bioactive compounds better than other solvents. Ethyl acetate extracts was able to inhibit *Staphylococcus aureus, Micrococcus luteus* and *Enterococcus faecalis* whereas chloroform extract of Tir12 isolate was able to inhibit *Proteus mirabilis* and *Bacillus cereus* better than other solvent extracts. The potent isolate Mah25 was characterized by 16S rRNA sequencing and the isolate was identified as *Streptomyces* sp. MAH25 (AB899158).

Keywords: Actinomycetes, Shore line soil, Chloroform, Ethyl acetate, *Streptomyces* sp. TIR12

1. INTRODUCTION

Screening of marine actinomycetes for novel bioactive secondary metabolites for microbial infections is gaining momentum in modern years Mohan and Krishnan (2012). Recently Bay of Bengal has been targeted as a prospective source of marine derived bacterial bioactive compounds by several researchers Anindita et al. (2008). Secondary

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metabolites produced by marine actinomycetes have unique chemical structures and it may form the basis for the synthesis of new drugs Renu et al. (2008). Undeniably among the actinomycetes, *Streptomyces* sp. produced about 75% of commercially and pharmaceutically valuable antibiotics Miyadoh (1993), Sujatha et al. (2005). Actinomycetes from terrestrial sources produce hundreds of antibiotics which are widely used at present Satish et al. (2012). There are differences among the organisms prevailing in terrestrial and marine environments because of the variations in physical, chemical and biological factors Kumar and Kannabiran (2010). Cultural characteristics such as growth on different media, the colour of mycelia, formation of soluble and melanin pigments, biochemical characteristics, sensitivity to antibiotics, utilization of carbon and nitrogen source has been used for the classification of *Streptomyces* sp. Dietz (1988), Shirling and Gottlieb (1966). However there are no reports regarding the screening, characterization and antibacterial activity of actinomycetes from coastal soil of Tiruchendur (Tamil Nadu, India). Therefore, an attempt is made to characterize and study the antibacterial activity of the isolated actinomycetes from shore line soil of Tiruchendur.

2. OBJECTIVES OF THE STUDY

The study is carried out to fulfill following objectives:

1. To screen the actinomycetes isolated from the shoreline soil of Tiruchendur.
2. To study the characteristics of selected actinomycetes by morphological, physiological and biochemical methods.
3. To assess the antibacterial activity of various solvent extracts of selected actinomycetes by Kirby Bauer well diffusion method.
4. To characterize the most potent actinomycete by 16S rRNA sequencing.
5. To study the restriction sites and secondary structure of the most potent actinomycete.

2.1. Hypotheses for the Study

The study is based on following hypotheses:

1. There is a significant difference in the morphological, physiological and biochemical characteristics of the isolates.
2. There is a significant difference between the solvents used for extracting the bioactive compounds for antibacterial activity.

2.2. Material and Methods

2.2.1. Screening of actinomycetes for antibacterial activity

Screening of actinomycetes was performed by agar well diffusion method Amit et al. (2011) against *Escherichia coli*, *Proteus* sp., *Pseudomonas aeruginosa* and *Bacillus subtilis*. 75µl of the actinomycetes culture (in stationary phase) were pipetted directly into well. The plates were incubated in upright position at 37^0C for 18 to 24 hours. After the incubation period, zones of inhibition were measured.

2.2.2. Characteristics of actinomycetes

Growth rate and colour of culture was observed by growing the actinomycete in twelve different medium. The physiological effect of culture conditions were studied by growing the actinomycete in Malt extract Yeast extract agar medium in different pH (5, 6, 7, 8, 9, 10, 11 and 12), temperature (10^0C, 15^0C, 25^0C, 37^0C, 45^0C and 60^0C). Their tolerance to sodium chloride was studied (0%, 0.5%, 1%, 1.5%, 2%, 3%, 5% and 10%) by incorporating different concentrations of sodium chloride in (MNA) Modified Nutrient agar medium Chacko and Ernest (2012). Gram staining was done to confirm the gram nature of actinomycetes Duraipandiyan et al. (2010). The mycelial colour and nature of mycelium were identified by cover slip culture Cross (1989) in which the culture was transferred to the base of the cover slip and it was buried in yeast extract malt extract agar medium (ISP2) at 28^0C for 10 days and observed under the microscope.

Antibiotic sensitivity pattern of actinomycetes was determined by growing a lawn culture of screened actinomycetes in Muller Hinton agar medium supplemented with antibiotic disc of Ampicillin, Chloramphenicol, Gentamycin, Methicillin, Penicillin G, Rifampicin, Tetracycline, Vancomycin and Kanamycin which were placed on the agar surface and incubated at 28^0C for 3 days. Tolerance towards Phenol, Crystal violet and Sodium azide were studied by agar well diffusion method, where the lawn cultures of screened actinomycetes were grown in Muller Hinton agar medium and the wells were made with sterile cork borer and 50µl of the Phenol/Crystal violet/Sodium azide were poured in the agar well and the plates were incubated at 28^0C for 3 days and the results were examined.

Carbon utilization was determined in petriplates containing ISP9 Basal medium in which the carbon sources like Lactose, Glucose, Mannitol, Glycerol, Sucrose, Xylose, Dextrose, Fructose, Galactose, Sorbitol, Starch, Inositol, Arabinose, Raffinose, Rhamnose and Maltose were added to the final concentration of 1%. The plates were incubated at 28^0C for 7 days and the results were observed. Nitrogen utilization was determined in petriplates containing the

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Table 1
Screening of actinomycetes

Isolate	*E.coli*	*Proteus* sp.	*Bacillus subtilis*	*Pseudomonas aeruginosa*
	Zone of Inhibition (mm)			
Tir4	0	0	0	0
Tir6	0	0	0	0
Tir 7	10	10	12	9
Tir 12	8	6	6	10
Tir 13	9	12	8	9
Tir14	0	0	0	0

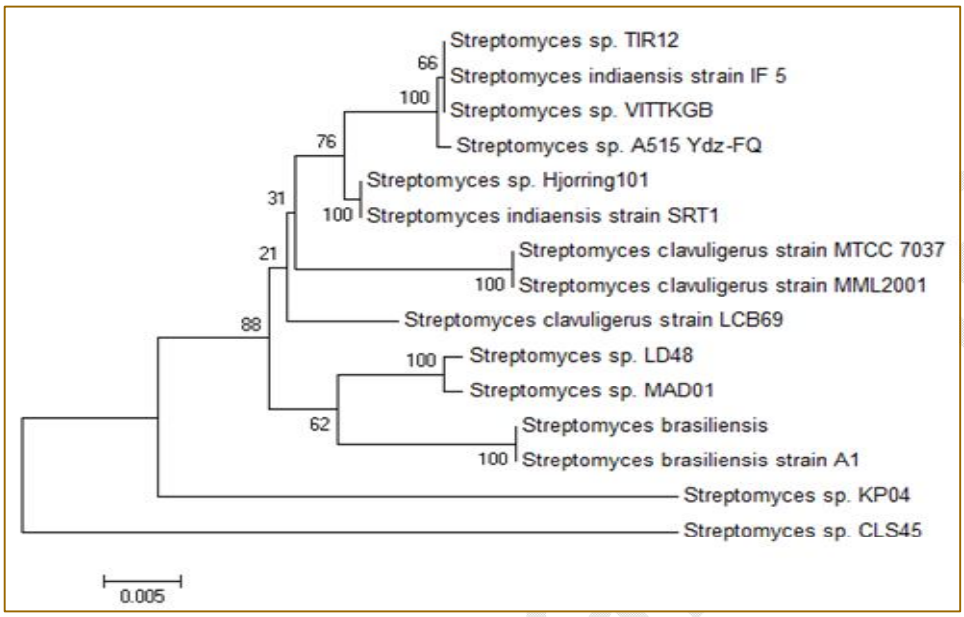

Figure 1
Relationships between *Streptomyces* sp. TIR12 and the members of the genus *Streptomyces* on rooted neighbour joining tree method based on 16S rRNA sequences. The numbers at the nodes of the phylogenetic tree indicates the level of bootstrap based on the analysis of 1000 resampled data sets. The scale bar indicates 0.005 substitutions per nucleotide position

basal medium Glucose-10g, $MgSO_4.7H_2O$-0.5g, $FeSO_4.7H_2O$-0.01g, K_2HPO_4-1g, NaCl-0.5g, Agar-20g, Distilled water-1000ml. Various nitrogen sources used in the study were Peptone, Ammonium nitrate, Yeast extract, Malt extract, Casein, Beef extract, Ammonium sulphate, Urea, Potassium nitrate, Sodium citrate, Sodium nitrate and Ammonium dihydrogen phosphate were added to the final concentration of 1%. The plates were incubated at 28^0C for 7 days and the results were monitored. Biochemical test such as Lipase hydrolysis, Urea hydrolysis, Cellulase, Phosphatase, Starch hydrolysis, Casein hydrolysis, Oxidase, Catalase, Nitrate reductase, Gelatin hydrolysis, Esculin hydrolysis, Xanthine hydrolysis, Hypoxanthine hydrolysis, Tyrosine hydrolysis, H_2S production, Citrate utilization and Indole production were done for the screened actinomycetes as recommended by International Streptomyces Project Baskaran et al. (2011).

2.2.3. Mass culturing of actinomycete and extraction of bioactive compounds

The actinomycete was inoculated in ISP2 broth and incubated at 28^0C in a water bath shaker at 200 rpm for 7 days. The culture along with the medium was centrifuged after the incubation at 5000 rpm for 10 minutes and the volume of the culture filtrate was added with equal volume of various solvents (viz., Ethanol, Ethyl acetate, Acetone, Hexane, Chloroform, Petroleum ether, Dichloro ethane and Methanol) separately and centrifuged at 5000 rpm for 10 minutes Sambamurthy and Ellaiah (1974). The supernatant was collected and stored for further analysis.

2.2.4. Antibacterial activity of crude extracts of actinomycete and commercial antibiotics against test bacteria

The antibacterial activities of crude extracts of various solvents of the antagonistic actinomycete were tested against the test organisms by Kirby Bauer agar well diffusion method. *Staphylococcus aureus, Micrococcus luteus, Neisseria mucosa, Bacillus cereus, Proteus mirabilis, Proteus vulgaris* and *Enterococcus faecalis* were obtained from MTCC, Chandigarh. 100µl of the extract was pipetted into the well. For all the bacteria, controls were tested in separate petriplates that consist of pure solvents instead of crude extract Parekh and Chand (2007). The antibacterial activity of the selected test bacteria were validated against commercially available antibiotic discs such as Kanamycin (30mcg) and Ampicillin (10mcg) by disc diffusion method. All the experiments were done in triplicate. The results were reported as Mean±Standard deviation. The results were analyzed statistically by Microsoft Excel 2010 (Roselle, USA).

2.2.5. Molecular characterization

16S rRNA analysis was performed using big dye terminator version 3.1 cycles sequencing kit and ABI 3130 Genetic Analyser (Yaazh Xenomics, Madurai). The unknown sequence of the isolate was compared by alignment against 16S

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Table 2
Growth characteristics of isolates in different medium

Medium	Growth rate	Colour	Growth rate	Colour	Growth rate	Colour
	Tir7		Tir12		Tir13	
Kuster's	Good	Dull white	Excellent	Dull white	Excellent	Pale yellow
Starch casein	Good	Dull white	Excellent	Pale white	Excellent	Creamy white
Nutrient	Good	Light yellow	Fair	Dull white	Excellent	White
ISP-1	Fair	Dull white	Good	Dull white	Excellent	White
ISP-2	Fair	Dull white	Excellent	White	Excellent	White
ISP-3	Good	White	Fair	Pale white	Excellent	White
ISP-4	-	-	Fair	Pale white	Fair	Creamy white
ISP-5	Fair	White	Excellent	White	Excellent	White
ISP-6	Excellent	White	Good	White	Excellent	White
ISP-7	Excellent	Pale white	Excellent	Creamy white	Excellent	Creamy white
Sea water	Excellent	Pale yellow	Excellent	White	Excellent	White
Marine	Excellent	Creamy white	Excellent	White	Excellent	Pale yellow

Table 3
Optimum physiological characteristics of isolates

Optimum parameter	Tir7	Tir12	Tir13
pH	7	7 to 10	7 to 10
Temperature	25^0C	25^0C	25 to 37^0C
Sodium chloride	1%	1.5 to 2%	0.5 to 1.5%

rRNA sequences available in the GenBank Database. The ~1.5 kb rRNA fragment was amplified using high fidelity PCR polymerase and the PCR product was sequenced bi-directionally using the forward primer and reverse primer Swetha and Valli (2012). The sequence data was aligned and analysed to identify the actinomycetes and its closest neighbours. The consensus 16S rRNA primers used in the reaction include 16S Forward primer 5'AGAGTTTGATCMTGGCTCAG3' and 16S Reverse primer 5'TACGGYTACCTTGTTACGACTT3'. According to the International Union of Biochemistry convention, R represents A/G nucleotide degeneracy, Y represents C/T nucleotide degeneracy, M represents A/C nucleotide degeneracy and W represents A/T nucleotide degeneracy Gowri and Asit (2010). Nucleotide sequences were compared with other 16S rRNA sequences, maintained by GenBank database, using the NCBI-BLAST program. The sequences were aligned by CLUSTAL W and the phylogenetic tree was constructed using MEGA5.1 Beta4 with the neighbour joining method. All these analysis were performed on a bootstrapped dataset containing 1000 replicates.

2.2.6. Prediction of secondary structure and restriction sites

The RNA secondary structure of the actinomycete was predicted using GeneBee online software by greedy method and the restriction sites of the 16S rRNA was analysed by NEB cutter.

3. RESULTS

The results of the preliminary screening were shown in Table 1 & Figure 1. The zone of inhibition was measured as a total diameter of the zone and the well diameter was subtracted from the total diameter. In the screening of actinomycetes Tir7, Tir12 and Tir13 were having antagonistic activity against the bacterial pathogens therefore, they were chosen for further studies.

The three isolates were white in colour and the isolates grew well in ISP6, ISP7, Marine agar and Sea water agar medium. Isolate Tir13 had grown well in all the twelve medium tested. Tir7 isolate failed to grow in ISP4 medium. The isolates did not produce melanoid and soluble pigments. The results of the growth characteristics were showed in Table 2.

The actinomycetes were gram positive, filamentous bacteria. Tir7 isolate was non motile and the mycelium was grey in colour. Tir12 and Tir12 were motile and the mycelium was dull white in colour. The optimum concentration for the growth of actinomycetes was given in the Table 3. Tir7 isolate was able to hydrolyze urease, oxidase, citrate and esculin. Tir12 isolate was able to hydrolyze urease, oxidase, amylase, protease, nitrate reductase and esculin whereas Tir13 isolate hydrolyze lipase, urease, catalase, oxidase, amylase, esculin and hypoxanthine. All the three isolates were resistant to phenol, crystal violet, sodium azide and Methicillin. Tir7 isolate was also resistant to Penicillin G. These three isolates were sensitive to Ampicillin, Chloramphenicol, Gentamycin, Rifampicin, Tetracycline, Vancomycin

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Table 4

Antibacterial activity of solvent extracts with commercial antibiotics of actinomycetes

	S.aureus	*M.luteus*	*N.mucosa*	*E.faecalis*	*P.vulgaris*	*P.mirabilis*	*B.cereus*
Zone of inhibition (mm)							
Isolate	**Ethyl acetate extract**						
Tir7	0	12±1	0	9.67±0.6	0	0	0
Tir12	4±0.6	14.67±0.6	0	13.67±0.6	0	0	1.67±0.6
Tir13	2.33±1	9.67±0.6	2.67±0.6	0	0	0	0
Isolate	**Chloroform extract**						
Tir7	0	0	0	0	4.67±0.6	0	4.67±0.6
Tir12	0	0	0	0	6.67±0.6	2.67±0.6	7.33±0.6
Tir13	0	0	0	0	14±1	0	5.33±0.6
Isolate	**Ethanol extract**						
Tir7	0	14.33±0.6	0	5.33±0.6	0	0	0
Tir12	0	6±1	0	8.33±0.6	0	0	0
Tir13	0	11±0	0	0	0	0	0
Isolate	**Methanol extract**						
Tir7	0	14.33±0.6	0	3±1	0	0	0
Tir12	0	13.33±0.6	0	7.33±0.6	0	0	0
Tir13	0	11.67	0	0	0	0	0
Isolate	**Dichloroethane extract**						
Tir7	0	0	0	0	5.67±0.6	0	4.67±0.6
Tir12	0	0	0	0	8±1	0	7.33±0.6
Tir13	0	0	0	0	13.67±1.15	0	5.33±0.6
Isolate	**Acetone extract**						
Tir7	0	12.67±0.6	0	3.33±0.6	0	0	0
Tir12	0	13.67±0.6	7.67±0.6	0	0	0	0
Tir13	0	13.67±0.6	0	0	0	0	0
Antibiotics	**Commercial antibiotics**						
Kanamycin	12±2	12.67±0.6	14.33±0.6	14±1	15.67±2.1	1.33±0.6	13.33±0.6
Ampicillin	13±1	7±1	31.67±1.15	35±2	11±1	9.33±2.31	12.33±1.53

and Kanamycin. The isolates utilized starch, glucose, dextrose, lactose, mannitol, galactose, mannose, inositol, glycerol, sucrose, fructose and maltose for its carbon source. These isolates were not able to grow in arabinose, rhamnose and raffinose. Peptone, casein, urea, sodium citrate, beef extract, malt extract, yeast extract, potassium nitrate, sodium nitrate were able utilized by the actinomycete as nitrogen sources. The isolates were not able to utilize ammonium sulphate, ammonium nitrate and ammonium dihydrogen phosphate as nitrogen sources.

The results of the antibacterial activity of various solvent extracts of the isolates and the antibacterial activity of the commercial antibiotics were given in Table 4. Hexane and Petroleum ether extracts did not exhibit any antibacterial activity against the bacterial pathogens. Ethyl acetate extract obtained from the isolate Tir12 showed significant antibacterial activity against gram positive and gram negative bacterial pathogens. Ethyl acetate extracts

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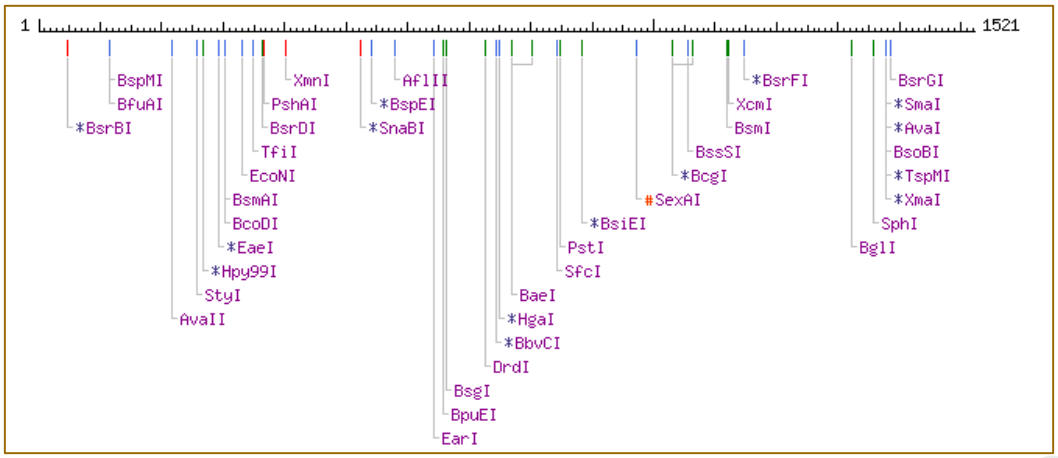

Figure 2

Restriction sites of the 16S rRNA sequence of *Streptomyces* sp. TIR12

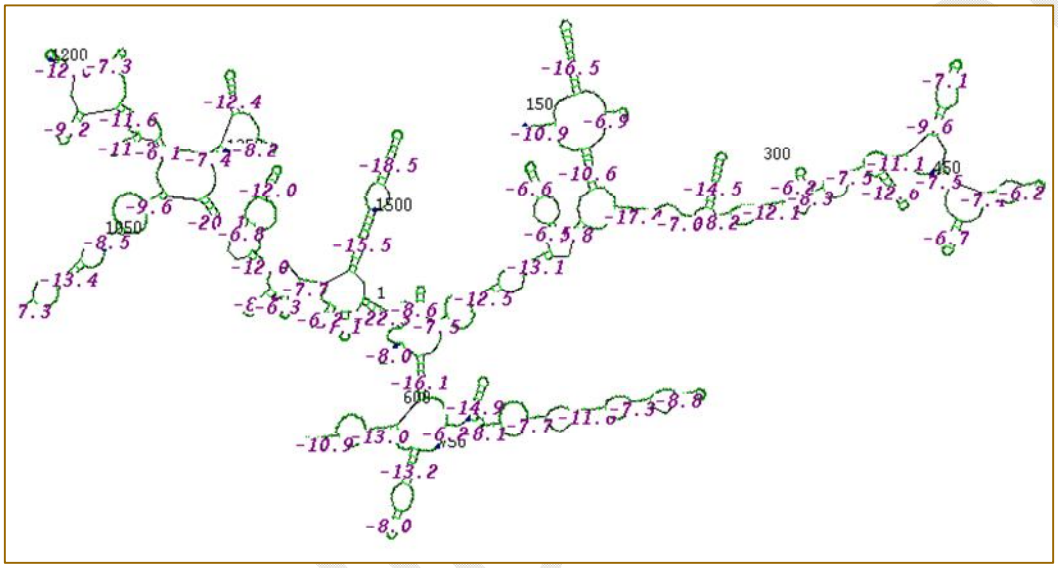

Figure 3

Secondary structure prediction of 16S rRNA sequence of *Streptomyces* sp. TIR12

was able to inhibit *Staphylococcus aureus, Micrococcus luteus* and *Enterococcus faecalis* whereas chloroform extract of Tir12 isolate was able to inhibit *Proteus mirabilis* and *Bacillus cereus* better than other solvent extracts. When comparing the antibacterial activity of extracts of actinomycete Tir12 with commercial antibiotics, the extracts showed better results against selected bacterial pathogens. The extract showed better activity than Ampicillin and Methicillin against *Micrococcus luteus*. The extract showed better antibacterial activity than Kanamycin against *Proteus mirabilis*. Chloroform extract of the actinomycete Tir13 showed significant antibacterial activity against *Proteus vulgaris* and it was better than Ampicillin.

The BLAST search of 16S rRNA sequences (1521 base pairs) of the actinomycete showed maximum of 99% similarity with *Streptomyces indiaensis* strain IF5 (FJ951435.1) and *Streptomyces* VITTKGB (GU358071.1). Based on the molecular taxonomy and phylogeny of the actinomycete, it was identified as *Streptomyces* sp. and it was designated as Streptomyces sp. MAH25. A neighbour joining tree based on 16S rRNA sequence of *Streptomyces* sp. TIR12 showed that, the isolate occupied a distinct phylogenetic position within the representatives of the Streptomycetes family. The nucleotide sequence of 16S rRNA gene partial sequence of *Streptomyces* sp. TIR12 was deposited in DDBJ under the accession number AB899158.

The prediction for the 16S rRNA sequence of *Streptomyces* sp. TIR12 showed the restriction sites for various enzymes such as Ava II, Sty I, Hpy99I, Eae I, BcoDI, Eco NI, TfiI, BsrDI, PshAI etc. (Figure 2). It showed GC and AT content of 55% and 45% respectively. The RNA secondary structure was predicted for the 16S rRNA sequence of *Streptomyces* sp. TIR12 was shown in Figure 3. It showed that the free energy of the secondary structure was -365.9Kkal/mol, threshold energy with cluster factor, conserved factor 2 and compensated factor 4 and conservativity is 0.8.

4. CONCLUSION

The potent isolate Tir12 was found to be *Streptomyces* sp. and it was designated as *Streptomyces* sp. TIR12. The free energy of the secondary structure was found to be -365.9Kkal/mol threshold energy with cluster factor, conserved factor 2 and compensated factor 4 and conservativity is 0.8. Ethyl acetate extracts of Tir12 was able to inhibit *Staphylococcus aureus, Micrococcus luteus* and *Enterococcus faecalis* whereas chloroform extract of Tir12 isolate was able to inhibit *Proteus mirabilis* and *Bacillus cereus* better than other solvent extracts. Ethyl acetate and Chloroform

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were found to be suitable for the extraction of antibacterial compounds from the culture filtrates. Further studies were needed to identify the bioactive compounds present in the culture filtrate of ethyl acetate and chloroform.

SUMMARY OF RESEARCH

1.The actinomycetes isolates were screened for their antibacterial activity and three actinomycetes isolates were selected for further study.
2.The morphological, physiological and biochemical characteristics were studied for the three isolates.
3.The three isolates were mass cultured and the bioactive compounds were extracted using eight different solvents and the antibacterial activity of the crude extracts were checked by Kirby Bauer well diffusion method.
4.Tir12 isolate was found to be the most potent actinomycete and the solvents ethyl acetate and chloroform extracted the bioactive compounds better than other solvents.
5. Tir12 was found as *Streptomyces* sp. TIR12 by 16S rRNA sequencing and the sequence were submitted in DDBJ and got the accession number AB899158.
6. The secondary structure of the RNA was predicted and the free energy was calculated by GeneBee online software.
7. The number of restriction sites and the GC content was studied by Neb cutter tool.

FUTURE ISSUES

The bioactive compounds of the potent actinomycete *Streptomyces* sp. TIR12 has to be identified, purified and characterize by analytical methods.

DISCLOSURE STATEMENT

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REFERENCES

1. Amit P, Imran A, Kailash SB, Tanushri C, Vidyottma S. Isolation and Characterization of actinomycetes from soil and evaluation of antibacterial activities of actinomycetes against pathogens. *International Journal of Applied Biology and Pharmaceutical Technology*, 2011, 2(4), 384-392
2. Anindita M, Subhas CS, Joydeep M. Distribution of actinomycetes, their antagonistic behavior and the physicochemical characteristics of the world's largest tidal mangrove forest. *Applied Microbiology and Biotechnology*, 2008, 80, 685-695
3. Baskaran R, Vijayakumar R, Mohan PM. Enrichment method for the isolation of bioactive actinomycetes from mangrove sediments of Andaman Islands, India. *Malaysian Journal of Microbiology*, 2011, 7, 26-32
4. Chacko VS, Ernest D. A comparative study on selected marine actinomycetes from Pulicat, Muttukadu and Ennore estuaries. *Asian Pacific Journal of Tropical Biomedicine*, 2012, 1827-1834
5. Cross T. Growth and examination of actinomycetes some guidelines. In Bergey's Manual of Systematic Bacteriology. Vol. 4. Williams and Wilkins company, Baltimore, 1989, 2340-2343
6. Dietz A. Biology and Actinomycetes, Japan Science Society Press, Tokyo, 1988, 203-209
7. Duraipandiyan V, Sasi AH, Islam VIH, Valanarasu, Ignacimuthu S. Antimicrobial properties of actinomycetes from the soil of Himalaya. *Journal de Mycologie Medicale*, 2010, 20, 15-20
8. Gowri S, Asit RG. *Pediococcus* sp. a potential probiotic isolated from Khadi (an Indian fermented food) and identified by 16SrDNA sequence analysis. *African Journal of Food Science*, 2010, 4, 597-602
9. Kumar S, Kannabiran K. Diversity and optimization of process parameters for the growth of *Streptomyces* VITSVK9 spp. isolated from Bay of Bengal, India. *Journal of Natural and Environmental Sciences*, 2010, 1(2), 56-65
10. Miyadoh S. Research on antibiotic screening in Japan over the last decade: A producing microorganisms approach, *Actinomycetologica*, 1993, 9, 100-106
11. Mohan KT, Krishnan K. Antimicrobial and antioxidant properties of marine actinomycetes *Streptomyces* sp. VITSTK7. *Oxidants and Antioxidants in Medical Science*, 2012, 1(1), 51-57
12. Parekh J, Chand S. In vitro antimicrobial activity of *Trpanatans* L. fruit extracted in different solvents. *African Journal of Biotechnology*, 2007, 6, 766-770
13. Renu S, Monisha K, Rup L. Bioactive compounds from marine actinomycetes. Indian Journal of Microbiology, 2008, 48, 410-431
14. Sambamurthy K, Ellaiah P. A new streptomycin producing neomycin (B and C) complex *Streptomyces marinensis*. *Hindustan Antibiotics*, 1974, 17, 24-28
15. Sathish KSR, Kokati VBR. In vitro antimicrobial activity of marine actinobacteria against multidrug resistance *Staphylococcus aureus*. *Asian Pacific Journal of Tropical Biomedicine*, 2012, 2(10), 787-792
16. Shirling EB, Gottlieb D. Methods for the characterization of *Streptomyces*. *International Journal of Systematic Bacteriology*, 1996, 16, 313-340
17. Sujatha P, Bapiraju KVVSN, Ramana T. Studies on antagonistic marine actinomycetes from the Bay of Bengal. *World Journal of Microbiology and Biotechnology*, 2005, 21, 583-585
18. Swetha S, Valli NC. Biogenesis of antibacterial silver nanoparticles using the endophytic bacterium *Bacillus cereus* isolated from *Garcinia xanthochymus*. *Asian Pacific Journal of Tropical Biomedicine*, 2012, 2(12), 953-959

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