World Inventia Publishers

Journal of Pharma Research

http://www.jprinfo.com/

Vol. 7, Issue 7, 2018 ISSN: 2319-5622 USA CODEN: JPROK3

Research Article

SECONDARY STRUCTURE PREDICTION AND RESTRICTION SITE ANALYSIS OF *STREPTOMYCES SP* ISOLATED FROM VALPARAI HILL STATION SOIL SITES

Vineeth. M [1] * and R. Ragunathan [2]

*[1] Assistant Professor, Department of Biotechnology, Rathnavel Subramaniam College of Arts and Science, Coimbatore -641402,Tamilnadu, INDIA.

[2] Director, Centre for Bioscience and Nanoscience Research, Coimbatore -641021, Tamilnadu, INDIA.*

Received on: 13-07-2018; Revised and Accepted on: 24-07-2018

ABSTRACT

In the current paper soil samples were collected from Valparai hil station sites to screen the diversity of actinomycetes. The potential actinomycetes were isolated by serial dilution and plating method on starch peptone agar media in the incubation of 7 days. After incubation morphological characterization was carried on the basis of shape and elevation characteristics on starch peptone agar and most dominative Streptomyces isolate were picked and carried for further inoculation. Streptomyces RRMVCBNR isolate was Phenotypic characterized on the basis of microscopic, colony morphology, biochemical and phenotypic studies. Which was further confirmed by genotypic studies based on 16S rRNA gene sequences followed by phylogenetic tree construction. 16S rRNA gene sequences of strain used in this study exhibited sequence similarity in the range of 99-100% with those of selected isolate and it was identified as Streptomyces RRMVCBNR. The sequences of 16S rRNA genes were deposited in genbank and received the accession number MG287121.The FASTA product was used to Secondary Structure prediction and Restriction site analysis of Streptomyces RRMVCBNR. Further activity of Streptomyces RRMVCBNR strain showed highest antibacterial activity against Staphylococcus aureus has been studied.

KEYWORDS: *Streptomyces RRMVCBNR, Starch peptone agar, FASTA.*

INTRODUCTION

The majority of actinomycetes is free living and found widely distributed in many natural environments including various soil. They are found worldwide in soil and are important in soil ecology [1, 2]. *Streptomycetes* are metabolically diverse and can "feed on" almost anything, including sugars, alcohols, amino acids, organic acids, and aromatic compounds. Ecologically, actinobacteria particularly, *Streptomyces spp* [3]. Are generally saprophytic, soil dwelling organisms that spend the majority of their life cycles as semi-dormant spores [4]. Soil is the most important habitat of *Streptomycetes* [5] .Grass vegetation or soil rich in organic matter contains the highest numbers of Streptomycetes. Most *Streptomyces* species prefer a neutral to mildly alkaline pH [6]. Most soils contain 10^4 to 10^7 colony-forming units of *Streptomycetes* representing 1 to 20% or even more of the total viable counts [7]. For decades, microbial natural products have been one of the major resources for discovery of novel drugs [8]. Among the potential sources of natural products, *Streptomyces* have been proven to be a prolific source with a surprisingly small group of taxa accounting for the vast majority of compounds discovered [9, 10]. Of the 22,000 known microbial secondary metabolites, 70% are produced by actinomycetes, and two thirds of them are contributed by the genus *Streptomyces* [11, 12].

Streptomyces are Gram positive, filamentous bacteria, with high G+C content (69-78%) in DNA exhibiting highly differentiated developmental cycle [13]. A whole array of hierarchic classification system has been used to define genera and suprageneric groups of actinomycetes but partial sequence analysis of 16S rRNA is the most significant. The characterization of actinomycetes has a great potential to assist in further investigation and exploitation of the organisms [14]. One of these molecular tools is the PCR amplification of variable region of genes encoding 16S rRNA (16S rDNA) by use of primers homologous to the conserved regions of the gene [15]. Subsequent electrophoretic separation of the PCR products in a polyacrylamide matrix, give comparable fingerprints of microbial communities [15]. Restriction endonuclease analysis patterns [16], secondary structure prediction [17] could be used for both to distinguish between the species of *Streptomyces* and to recognize variation within the species. Thus, the aim of this study is to identify actinomycetes from the soil, investigate for their characterization of phenotypic, genotypic, Secondary Structure prediction, Restriction site analysis finally to check bioactivity conditions against clinical pathogens associated with antibiotic resistance.

***Corresponding author:**

Vineeth. M

Assistant Professor, Department of Biotechnology, Rathnavel Subramaniam College of Arts and Science, Coimbatore -641402,Tamilnadu, INDIA.

** E-Mail: sureshbioteck@gmail.com*

DOI: https://doi.org/10.5281/zenodo.1322956

MATERIAL AND METHODS

Soil Sampling and transport:

Soil samples were collected from different sites of Valparai (latitude 10.37°N and longitude 76.97°E. 3,914 feet) Taluk and hill station in the Coimbatore district of Tamil Nadu, India. Samples were collected by inserting a sterilized polyvinyl corer into the sediments. The corer was sterilized with alcohol before sampling at each location. Each collection was made from 4 cm depth of the surface of ground. These samples were placed in sterile poly bags, sealed tightly, and transported immediately to the laboratory. These soil samples were air-dried for 3-4 h at 45°C, crushed, and sieved prior to use for further.

Isolation of *Sterptomyces:*

Starch agar medium (Starch 2g, Peptone 0.5g, $NaNO_3$ 0.2g K_2HPO_4 0.5g, $MgSO_4$ 0.5g, D.W 100 ml) was prepared and sterilized at

121°C in 15 lbs pressure for 15 min. Then it was supplemented with streptomycin 30 µg/l to prevent the bacterial growth [9]. The medium was poured into the sterile Petri plates. The collected soil samples were diluted upto 10-6 and 0.1 ml of the diluted samples was spread over the starch agar medium. The inoculated plates were incubated at 30°C for 7 days. Replicates plates were maintained for each dilution. After incubation, the dominative actinobacterial colonies were purified by streak plate technique, sub-cultured and stored at 4°C on SPA slants for further.

Colony characterization of *Streptomyces:*

Colony morphology of the purified *Streptomyces* isolates on SPA medium were recorded with respect to colour of aerial spore mass, size and nature of the colonies, colour on the reverse side and diffusible pigmentation.

Phenotypic characterization of isolate:

The biochemical characterization of Indole, MR, VP, Citrate, H2S Urease, Oxidase test, gelatin hydrolysis, starch hydrolysis, urea hydrolysis, acid production from different sugars, motility test, triple sugar iron (TSI) agar test, with ***Streptomyces*** RRMVCBNR was performed and further carried genotypic characterization.

Genotypic identification of the isolate:

Isolation of chromosomal DNA:

Dominative isolate of *Streptomyces* was grown upto the late exponential phase in Starch Peptone broth incubation at 30°C, after 7 days cells were harvested and washed with Tris EDTA buffer to DNA isolation. Total genemic DNA were isolated by resuspending the cells and add 6 ml lysis buffer and incubated for 30-80 min at 37°C respectively, after that add 500 µl of 2 M NaCl solutions. Entire suspension was agitated on a vortex mixer until the cell suspension became semi-transparent. After that cells were lysed by the addiing of 1.2 ml of 10% SDS solution. The lysates were incubated for 15-30 min at 65°C. After addition of 2.4 ml of 5 M potassium acetate, the solution was mixed and left in ice box for 20 min. the precipitate was removed by centrifugation for 30 min at 6,000 rpm and the volume of the supernatant was adjusted to 8 ml. DNA was recovered by precipitation with 2 volume of isopropanol. The precipitate was dissolved in 600 µl/g of 10 mM Tris/1 mM EDTA (pH 8.0) and the aqueous phase was transferred to a 1.5 ml micofuge tube. Subsequently, 75 µl 3 M sodium acetate and 500 µl isopropanol were added and the solution was centrifuged for 30 sec to 2 min. Step 6- the precipitate was washed with 70% cold ethanol, dried and dissolved in 100 µl TE buffer. Collected DNA was used for PCR amplification with primer (5"-AGAGTTTGATCCTGGCTCAG 3"), downstream primer, 10 µl (5"-AGGGCTACCTTGTTACGACTT 3").

NCBI-BLAST- Sequences accession number:

Then PCR product was sent to sequencing by automated sequenced method. The same primers as reported above were used for sequencing. Further, NCBI-BLAST www.ncbi.nlm.nih.gov/ blast was used to compare the sequence similarity of isolated *Sterptomyces* strain with reference strain. The 16S rRNA a sequence of *Sterptomyces* was deposited in NCBI and the sequences accession number was obtained. The bootstrapped data set was used to build the phylogenetic tree by using the phylogeny fr.

Secondary structure prediction of 16S rDNA Streptomyces:

The secondary structures of 16S rDNA of *Streptomyces* RRMVCBNR **(**MG287121) was predicted using the bioinformatics tools available in online RNAWebSuite/RNAfold.

16S rDNA Sterptomyces of Restriction site analysis:

The restriction sites in 16S rDNA of *Streptomyces* RRMVCBNR **(**MG287121) was analysed using NEB cutter programme version 2.0 and energy dot plot analysis by http://rna.tbi.univie.ac.at.

***Sterptomyces* extract:**

Preparation of actinobacterial extract was done by submerged fermentation. *Streptomyces* RRMVCBNR isolate was taken in 50 ml of starch peptone broth in a 250-ml-capacity conical flask under sterile conditions and incubated at 30°C for 7 days at 150 rpm rotation. After fermentation, the medium was centrifuged at 10,000 rpm for 10 min to remove cells and debris and harvested for fermented broth and react with ethanol. Resultant fermented broth was used for further.

Screening of *Streptomyces* extract against URO pathogens:

The multi-drug resistant strains of uropathogens such as *Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi*, *E coli* and *Streptococcus pneumoniae,* were obtained from Microbiology unit, CBNR, Coimbatore, Tamilnadu.

Antimicrobial activity was determined by Agar well diffusion method. The isolated strains were spread across the Muller Hinton Agar (MHA) plates. The plates were incubated at 28°C for 48 hrs. After observing the fine growth of the strain, the 24 h cultures of uropathogens were load *Streptomyces* extract. All plates were incubated at 37°C for 24 h. After 24 h, the activities were observed based on the zone of inhibition.

RESULT

S*treptomyces* have been intensively studied in several under explored soil habitats in various parts of the Tamilnadu state in the last few years. Yet, there is no report regarding isolation of *Streptomyces* from valparai, Coimbatore, Tamilnadu. In the present work among dominative *Streptomyces* strains were isolated two sites from soil samples (Fig. 1) based on the colony morphology was differentiated. Colours of aerial spore mass of the isolates were categorized into 4 groups including white, grey, pink and brown series on Starch Peptone Agar. Among these 4 groups, most of the isolates were produced pink colour series than white, grey and brown series (Table. 1 Fig. 2).

Further, many of the isolates produced pink coloured substrate mycelium (reverse side colour) on SPA medium after seven days of incubation at 28°C. Based on the colony and microscopic (sporopore) morphology, most of the isolates (60%;n=30) belonged to the genus *Streptomyces* Therefore, an attempt has been made to isolate the *Streptomyces* from this unexplored region in order to find novel species (Table 2)

Cultural characteristics of the isolate were studied with Starch Peptone Agar media. Eight of the isolates produced pink, white and grey coloured spore mass and white, brown and yellowish reverse side in most of the media tested.

Totally 4actinobacterial strains were isolated based on the gram staining and colony morphology. All the isolates were found to be positive in gram staining and had different morphological structures. The biochemical properties of dominative *Streptomyces* isolate RRMVCBNR 1 were recorded. The dominative isolate was streaked on SPA plate and maintained (Fig. 3).

Among the various biochemical characteristics studied, positive result were observed with potential isolate *Streptomyces* RRMVCBNR 1 in production of Indole, MR, citrate , urease, oxidase, starch hydrolysis ,Acid production from different sugars, motility, TSI,VP, gelatin hydrolysis and Urea hydrolysis were observed (Table 3).

The isolated *Streptomyces* RRMVCBNR, genomic DNA was isolated with the DNA extraction method, amplified using the polymerase chain reaction and purified. Using the primers, the Size of fragments was obtained by amplification of 16s r-RNA region *Streptomyces* RRMVCBNR 1 -100bp.

The isolated DNA was respectively amplified and sequenced (16S rRNA gene sequencing). The aligned sequences were regions of local similarity with known sequences in the Genbank database using nucleotide BLAST at the National Center for Biotechnology Information (https://blast.ncbi.nlm.nih.gov/Blast). The isolated species were identifying based on homology scores with known species. Phylogenetic trees were constructed with robust phylogenetic tree software (Fig. 4).

In the phylogenetic tree, *Streptomyces* RRMVCBNR 1 was clustered together as one clade segments corresponding to an evolutionary distance of 0.01are shown with bars. Numbers above branches are bootstrap values showing greater than 90% (Fig. 4)

The secondary structure of 16S rRNA gene of *Streptomyces* RRMVCBNR (MG287121) showed 125 stems in their structure. However, the isolate was similar in energy thresh hold, cluster factor, conserved factor, compensated factor, conservativity, part of sequence, greedy parameters and treated sequence as indicated by RNA fold web server software (Fig. 5).

The restriction sites found in both *Streptomyces* RRMVCBNR (MG287121). Totally, 47 restriction enzyme sites were observed

however, the cleavage sites and nature of restriction enzymes. The GC,AT contents of were found to be 58% and 42% respectively (Fig. 6).

In the primary screening of antimicrobial activity against positive results *Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi*, *E coli* and negative against *Streptococcus pneumonia.*

The fermented broth containing antimicrobial compounds of selected potential *Streptomyces* RRMVCBNR (MG287121) were extracted with ethanol solvents. The extracted compounds were assessed for their antimicrobial ability against positive results *Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi* , *E coli*. The zone of inhibition was more sensitive in *Staphylococcus aureus* (Table 4).

Fig. 1: Panoramic view of sampling sites 1-2

Table No. 1: Colonies were isolated from Soil samples from hills

Area of collection	Dilution factor	Colony appearance	Number of colonies
Site 1	10^{-5} to 10^{-9}	Small white +Very small + Milky white colony	10+5+4
		Irregular white rough + Small creamy white	4+4
		Small dot like + Whitish with zone like appearance	4+2
		Pink coloured + Small white	4+5
Site 2	10^{-5} to 10^{-9}	Big colonies with dot like appearance at centre	6+5
		Small grey irregular + Irregular branched	9+11
		Big grey	3+2+2
		Big pink colonies + Radial spokes like colonies	10+3

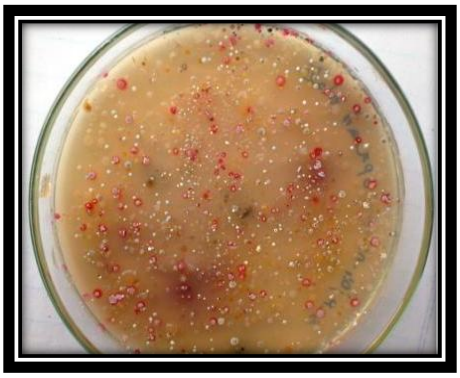


Fig. 2: Plates with Actinomycetes isolates

Table No. 2: Cultural and morphological characteristics of actinomycetes isolates

Isolate Name	Colony morphology		Cell -Microscopic Observation			
	Colour of aerial spore mass	Colour of reverse side	Shape	Aerial Mycelium	Substrate Mycelium	Colour of aerial spore mass
***Streptomyces* 1**	Pink	Pink	Spherical	Pink	Pink	Pink
Other isolate	Grey	Creamy white	Spherical	Grey	Light grey	Grey
Other isolate	Brown	Pale yellow	Ovoid	Brown	Yellowish brown	Brown
Other isolate	White	White	Ovoid	White	Milky white	White

Fig. 3: *Streptomyces* **isolate RRMVCBNR 1 steaked in SPA plate**

Table No. 3: Biochemical characteristics of the isolate *Streptomyces* RRMVCBNR 1

Isolate/Test	Indole/ MR/VP	Citrate/ H2S Urease	Oxidase test/ gelatin hydrolysis	starch hydrolysis/ urea hydrolysis	Acid production from different sugars	Motility test/ triple sugar iron
RRMVCBNR 1	+ve +ve +ve	+ve +ve	+ve +ve	+ve +ve	+ve +ve	+ve +ve

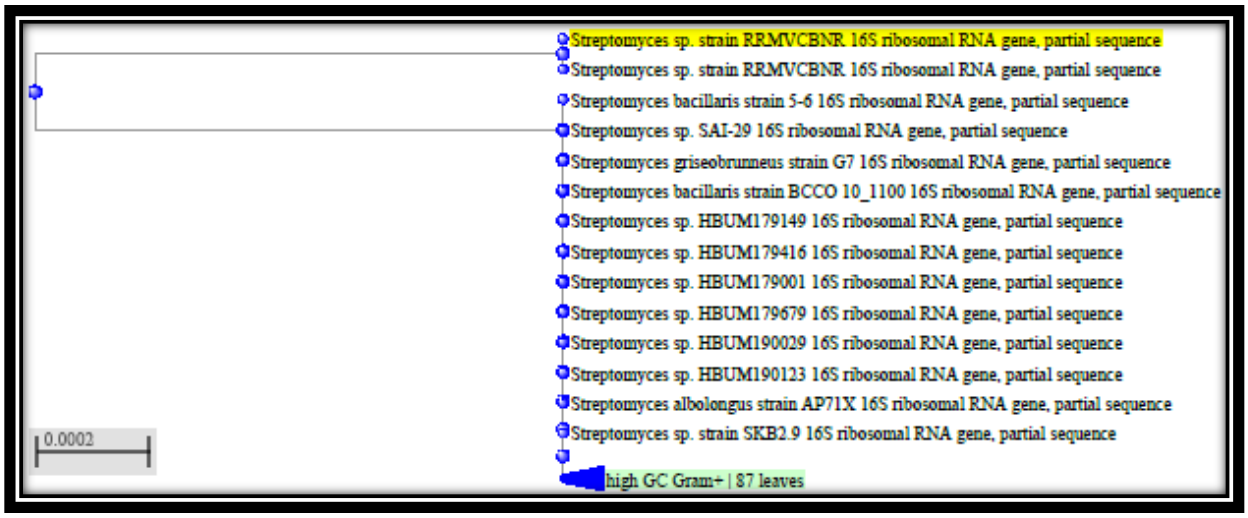
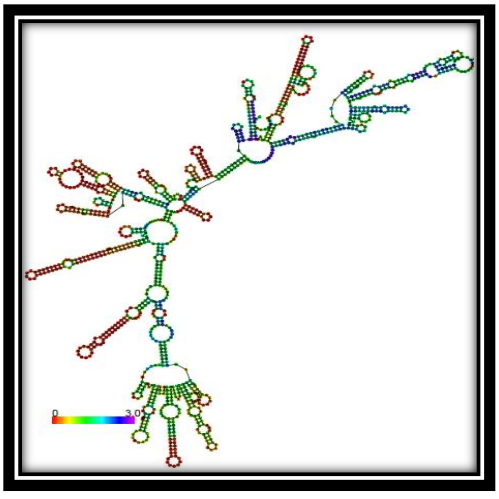
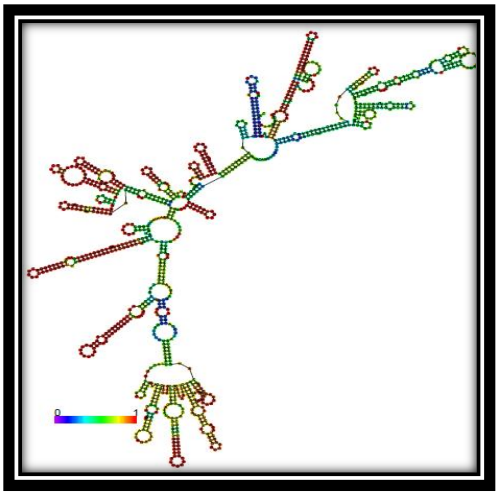


Fig. 4: Phylogenetic tree showing phylogenetic position of the isolates





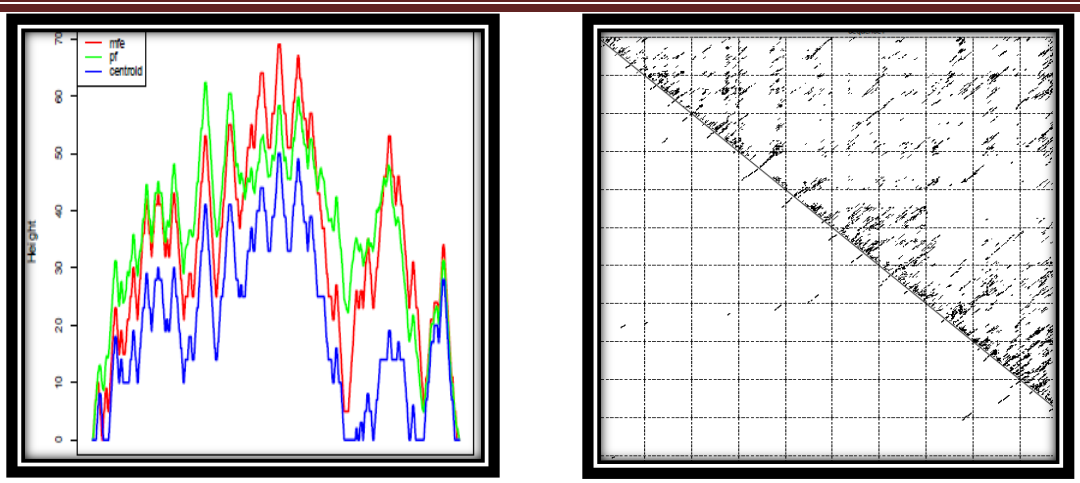


Fig. 5: Secondary structures and Energy dot plot of 16S rDNA RRMVCBNR 1

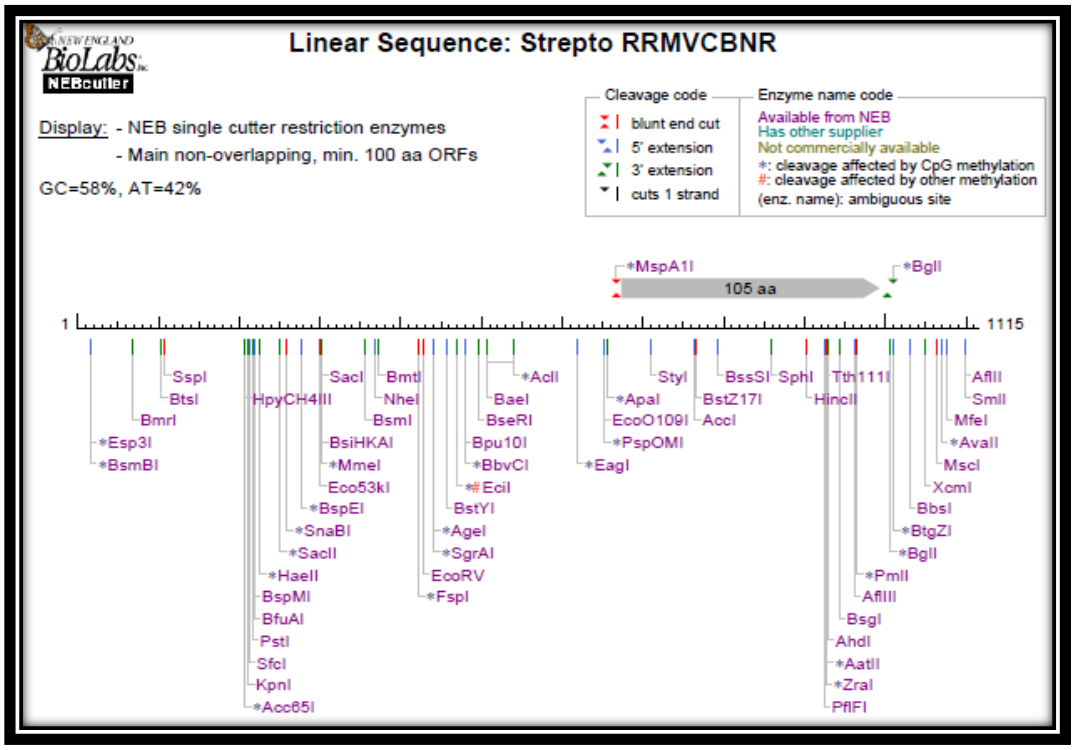


Fig. 6: Restriction sites and GC content for *Streptomyces* RRMVCBNR

Table No. 4: Antimicrobial efficacies of *Streptomyces* RRMVCBNR

Name of the isolate /Solvent	Diameter of inhibition zones (mm)				
	Bacillus subtilis	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Salmonella typhi*	*E. coli*
***Streptomyces* RRMVCBNR (MG287121) Ethanol**	12	18	11	12	11

DISCUSSION

In previous study *Streptomyces* spp. was isolated from rhizospheric soils of Ngaka Modiri Molema District, North West Province, South Africa [18]. The isolation of diverse and novel actinobacteria provides a theoretical guide for the exploitation and utilization of actinobacterial resources. The cultural and molecular identification of the potent actinobacterial isolates was carried out by amplifying the 16S rDNA and sequenced. Further, computational analysis including BLAST search and phylogenetic analysis of 16S rDNA confirmed the isolate as members of the genus *Streptomyces* with 89-100% sequence similarity [19-21]. In this study isolation of actinobacteria from valparai, Coimbatore, Tamilnadu. Among 4 actinobacteria strains were isolated from soil samples based on the colony morphology was differentiated. Among these 4 groups, most of the isolates were produced pink colour series than white, grey and brown series.

In previous study genus *Streptomyces*, a widespread group in phylum Gram positive actinobacteria, has received much attention owing to its ecological versatility, and ability to produce a rich array of bioactive metabolites. Its high environmental adaptability might be attributable to its genome dynamics, which can be estimated through comparative genomic analysis targeting microorganisms with close phylogenetic relationships but different phenotypes (Li et al., 2013).It is also evident that 16S rRNA gene sequencing has played a vital role in the identification of actinobacteria by many. [22-24].

In this study isolate *Streptomyces* RRMVCBNR in positive results production of Indole, MR, citrate, urease, oxidase, starch hydrolysis, Acid production from different sugars, motility, TSI and VP, gelatin hydrolysis and Urea hydrolysis. In previously Biochemical characterization of *Streptomyces Spp.* [25-27] Distinct variation in the secondary structure, G+C composition, presence of restriction enzymes sites in 16S rRNA gene sequence of isolates showed molecular level specificity of each and every individual isolates[28-30]. For the development of universal identification system of not only actinobacteria, but all microorganisms, a polyphasic taxonomic approach utilizing morphological, biochemical, physiological, cultural, ecological and molecular characteristics will help taxonomists for the development of meaningful taxonomic identification system. In previously taxonomic identification carried by many [31-33].

In the course of screening for novel antimicrobial substances (antibiotics) from soil samples, Out of 4 actinobacteria found to have antimicrobial activity in preliminary screening, *Streptomyces* RRMVCBNR (MG287121) was selected based on their zone of inhibition against *Staphylococcus aureus*. In previously antimicrobial activity were discussed by many others author. [34-36].

The author reported that restriction endonuclease analysis and secondary structure prediction patterns could be used for both to distinguish between the species of *Streptomyces* and to recognize variation within the species.

CONCLUSION

It was concluded that *Streptomyces* RRMVCBNR (MG287121) are omnipresent, they differed in their phenotypic and genotypic characterization depending on the physico-chemical properties and other nutrients of the habitats and also the isolate of *Streptomyces* varied in their antimicrobial activities on the other hand most of the isolates showed morphological homology and phenotypic characteristics expressed much variations within the genus/species, therefore the genotypic characterization such as 16S rRNA gene is a tool to find out its phylogeny, elucidating the secondary structure of rRNA and in the analysis of restriction sites and GC contents.

ACKNOWLEDGEMENT

The authors would like to thank Centre for Bioscience and Nanoscience Research, Coimbatore for valuable support. The authors are grateful to the Principal and Head, Department of Biotechnology Rathnavel Subramaniam College of Arts and Science, Coimbatore - 641402 for technical assistance and support.

REFERENCES:

1. Meyer J. Nocardiopsis, a new genus of the order Actinomycetales. Int J Syst Bacteriol **1976**;26:487-493.
2. Rodrigues de Melo, Gabriela Felix Persinoti , Douglas Antonio Alvaredo Paixaoa, Fabio Marcio Squinaa, Roberto Rullera, Helia Harumi Satob. Draft genome sequence of Streptomyces sp. strain F1, a potential source for glycoside hydrolases isolated from Brazilian soil. Braz J Microbiol **2017**;48:612-614.
3. Anuprita A, Deshmukh, Narendra N, Vidhale. Characterization of Novel Antibacterial Actinomycetes Strain N8 from Saline Soil of Vidarbha Region. Int J Life Sci Biotech Pharm Res **2015**;4(1):1-4.
4. Annika Rottig, Ewelina Atasayar, Jan Philipp Meier-Kolthoff, Cathrin Sproer,Peter Schumann,Jennifer Schauer, Alexander Steinbuchel. Streptomyces jeddahensis sp. nov., an oleaginous bacterium isolated from desert soil. Int J Syst Evol Microbiol **2017**;67:1676-1682.
5. Bouras N, Meklat A, Zitouni A, Mathieu F, Schumann P, Sproer C. Nocardiopsis algeriensis sp. nov an alkalitolerant actinomycete isolated from Saharan soil. Antonie Van Leeuwenhoek **2015**; 107:313-320.
6. Tahsin Bennur, Ameeta Ravi Kumar, Smita Zinjarde, Vaishali Javdekar. Nocardiopsis species: Incidence, ecological roles and adaptations. Microbiol Res **2015**;174:33-47.
7. Bhuneshwari Sahua, Roymon MG. Review on current techniques in isolation and characterization of Streptomyces from soil. Ind J Sci Res **2017**;13(2):226-232.
8. Meena B, Rajan LA, Vinithkumar NV, Kirubagaran R. Novel marine actinobacteria from emerald Andaman and Nicobar Islands: a prospective source for industrial and pharmaceutical byproducts. BMC Microbiol **2013**;13:145-146.
9. Meklat A, Sabaou N, Zitouni A, Mathieu F, Lebrihi A. Isolation, taxonomy, and antagonistic properties of halophilic actinomycetes in Saharan soils of Algeria. Appl Environ Microbiol **2011**;77:6710-6714.
10. Bantanahal Talari Vishwanatha, Girish Babu K, Padmashri, Swathi B Malagi, Chethan J. Dandin, Sreenivasa Nayaka. Isolation, Identification and Characterization of Streptomyces sp. SN-2. Biosci Biotech Res Asia **2017**;14(4):1401-1407.
11. Srinivasan M C, Laxman RS, Deshpande M V. Physiology and nutrition aspects of actinomycetes - an overview. World J Microb Biotechnol **1991**;7:171-184.
12. Hozzein WN, Goodfellow M. Nocardiopsis arabia sp. nov., a halotolerant actinomycete isolated from a sand-dune soil. Int J Syst Evol Microbiol **2008**;58:2520-2524.
13. Tian S, Yang Y, Liu K, Xiong Z, Xu L, Zhao L. Antimicrobial metabolites from a novel halophilic actinomycete Nocardiopsis terrae YIM 90022. Nat Prod Res **2014**;28:344-346.
14. Fatemeh Alzamoli. Isolation and characterization of Streptomyces species against vancomycin resistant enterococcus (VRE) from agricultural soils of Qom, Iran. Biosci Biotech Res Comm **2017**;10(2):220-224.
15. Jie Li, Jian Yang, Wen-Yong Zhu, Jie He, Xin-Peng Tian, Qiong Xie,Si Zhang , Wen-Jun Li. Nocardiopsis coralliicola sp. nov., isolated from the gorgonian coral, Menella praelonga. Int J Syst Evolu Microbiol **2012**;62:1653-1658.
16. Hotam S. Chaudhary, Jayprakash Yadav, Anju R. Shrivastava, Smriti Singh, Anil K Singh, Natrajan Gopalan, Antibacterial activity of actinomycetes isolated from different soil samples of Sheopur (A city of central India). **2013**;4(2):118-123.
17. Ravi Kumara, Smita Zinjardea, Vaishali Javdekarb, Tahsin Bennura, Ameeta. Nocardiopsis species: Incidence, ecological roles and adaptations. Microbiol Res **2015**;174:33-47.
18. Alaa-El Din MS Hosny, Donia H Sheir, Nagwa A Abdelallah, Dina HM Amin, Ahmed I El-Diwany, Nayera A M Abdelwahed, Ahmed A El-Beih, Adyary Fallarero ,Pia M Vuorela. Isolation of Streptomyces sp. producing antifungal agent. Curr Sci Int **2015**; 4(3):259-263.
19. Chen YG, Cui XL, Kroppenstedt RM, Stackebrandt E, Wen ML, Xu LH. Nocardiopsis quinghaiensis sp. nov isolated from saline soil in China. Int J Syst Evol Microbiol **2008**;58:699-705
20. Shade A, Hogan CS, Klimowicz AK, Linske M, McManus PS, Handelsman J. Culturing captures members of the soil rare biosphere. Environ Microbiol **2012**;14:2247-2252.
21. Santhoshkumar S, Nagarajan N. AM fungal association in the Rhizosphere soil of some Pteridophytic plant species in Valparai Hills, Western Ghats of Tamilnadu, India. Int J Life Sci **2014**;3: 201-206.
22. Gayathri A, Madhanraj P, Panneerselvam A. Diversity, Antibacterial Activity And Molecular Characterization of Actinomycetes Isolated From Salt Pan Region of Kodiakarai, Nagapattinam DT. Asian J Pharm Tech **2011**;1(3):79-81.
23. Ali Mohammed Abdullah Bawazir1, Ahmed Al-Haddad, Abdullah AL-Mahdi, Manjula Shantaram. Actinomycetes from Mountains of Hadhramout-Yemen. Int J Curr Microbiol App Sci **2017**;8: 3521-3530.
24. Jeffrey LSH, Sahilah AM, Son R. Tosiah S. Isolation and screening of actinomycetes from Malaysian soil for their enzymatic and antimicrobial activities. J Trop Agric Fd Sc **2007**;35(1):159–164.
25. Virali MS, Vennila S, Vignesh S, Vikash K,Vijayakumar R, Panneerselvam A. Antagonistic activity of actinomcyetes isolated from Marudhamalai Hills, Western Ghats. J Soil Biol Ecol **2007**;27(1):19-25.
26. Arifuzzaman M, KhatunM R, Rahman H. Isolation and screening of actinomycetes from Sundarbans soil for antibacterial activity. Afr J Biotechnol **2010**;9:4615-4619.
27. Deepa S, Panneerselvam A, Dhanasekaran D, Thajuddin N, Vijayakumar R. Diversity and antimicrobial potential of

actinobacteria from saltpan environment. Global Ad Res J Microbiol **2010**;1:140-148.
28. Kavitha A, Vijayalakshmi M, Sudhakar P, Narasimha G. Screening of actinomycete strains for the production of antifungal metabolites. Afr J Microbiol Res **2011**;4:27-32.
29. Sunita C, Mesta Onkarappa R. Characterization and Antimicrobial Potential of Marine Actinomycetes from Estuaries of Uttara Kannada District, Karnataka. J Drug Deliv & Therapeu **2017**;6:27-37
30. Mythili B, Ayyappa Das MA. Studies on antimicrobial activity of Streptomyces spp. isolates from tea plantation soil. Res J Agri Sci **2011**;2:104-106.
31. Nithya B, Ponmurugan P. Studies on actinomycetes diversity in Eastern Ghats (Yercaud Hills) of Southern India for secondary metabolite production. Int J Agri Res **2014**;7:152-159.
32. Mukesh Sharma, Pinki Dangi, Meenakshi Choudhary. Actinomycetes: Source, Identification, and Their Applications. Int J Curr Microbiol App Sci **2014**;3:801-832
33. Joseph Devadass B, Gabriel Paulraj M, Ignacimuthu S, Agastian Simiyon Theoder P , Al Dhabi NA. Identification of Antimicrobial Compounds from *Streptomyces* sp. Isolated from Western Ghats Soil in Tamil Nadu. EC Microbiol **2017**;1:222-231.
34. Basavaraj KN, Chandrashekhara S, Shamarez AM, Goudanavar PS, Manvi FV. Isolation and morphological characterization of antibiotic producing actinomycetes. Trop J Pharm Res **2010**;9: 231-236.
35. Monisha K, Renu S. Selective isolation of rare actinomycetes producing novel antimicrobial compounds. Int J Adv Biotechnol Res **2011**;2:357-375.
36. 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. J Med Mycol **2007**;17:242-249.

How to cite this article:

Vineeth. M and R. Ragunathan. SECONDARY STRUCTURE PREDICTION AND RESTRICTION SITE ANALYSIS OF *STREPTOMYCES SP* ISOLATED FROM VALPARAI HILL STATION SOIL SITES. J Pharm Res 2018;7(7):160-166.
DOI: https://doi.org/10.5281/zenodo.1322956

Conflict of interest: The authors have declared that no conflict of interest exists.

Source of support: Nil