IJBPAS, July, 2022, 11(7): 3220-3234

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)
'A Bridge Between Laboratory and Reader'

www.ijbpas.com

STUDIES ON ISOLATION, IDENTIFICATION AND ANTIMICROBIAL POTENTIAL OF STREPTOMYCES CINNAMONENSIS VLCH-1 FROM MANGROVE HABITATS

CH. CHIRANJEEVI[1] AND PROF. M. VIJAYALAKSHMI[2*]

1: Research Scholar, Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur - 522510, Andhra Pradesh, India

2: Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur - 522510, Andhra Pradesh, India

[*]**Corresponding Author: Dr. Muvva Vijayalakshmi: E Mail: muvvavijayalakshmi77@gmail.com**

Received 16th June 2020; Revised 24th July 2020; Accepted 29th Nov. 2021; Available online 1st July 2022

https://doi.org/10.31032/IJBPAS/2022/11.7.6226

ABSTRACT

Searching for novel antibiotics is the need of the hour to combat multidrug resistant pathogens. Hence, an attempt was made to isolate and identify promising antimicrobial metabolite producing actinomycetes from mangrove sediments of Machilipatnam, Andhra Pradesh, India along with the study of their antimicrobial potential. The samples collected from mangrove habitats were pretreated with calcium carbonate, diluted and plated on yeast extract malt extract dextrose (YMD) agar medium to isolate actinomycetes. Over all 10 strains designated as VLCH-1 to VLCH-10 were isolated and screened for their antimicrobial activity. Among them, one strain VLCH-1 which exhibited high antimicrobial potential was identified as *Streptomyces cinnamonensis* VLCH-1 based on polyphasic taxonomy including micro-morphological, cultural, biochemical, physiological and molecular methods (Genbank accession number: MZ951165). The growth pattern of the isolate was studied by culturing it in YMD broth. The culture broth extracted with ethyl acetate was tested for antimicrobial activity and found to be active against pathogenic Gram-positive, and Gram-negative bacteria as well as fungi. It was evident from the

present study that mangrove habitats of Machilipatnam serve as a good source for potent actinomycetes possessing broad spectrum antimicrobial activity.

Keywords: Mangrove actinomycetes, Phylogenetic study, *Streptomyces cinnamonensis* VLCH-1, Antimicrobial activity

INTRODUCTION

The extreme biospheres such as deep oceans, deserts and mangroves are the natural resources for novel pharmaceutical compounds. Nature acts as a prominent reservoir for new and novel therapeutics. Among them, mangroves are unique woody forests and also called as tidal forests, coastal woodlands or oceanic rain forests as they grow at the interface between land and sea in tropical and subtropical latitudes **[1, 2]**. They are distributed in 112 countries and territories. Remarkably India is one of the largest sub-continent with huge diversified mangrove geography, located within the tropical and subtropical belts.

The mangrove environment such as geographical location, pH, temperature, salinity fluctuations, moisture and nutrient differs greatly in different regions so that mangrove microbial communities are diverse, unique and valuable [**3, 4]** which produce different types of fascinating and structurally complex natural products with biological activity. Due to the presence of unique environment, mangrove forests support different groups of microorganisms, including bacteria, fungi and actinomycetes. Especially, actinomycetes possessing immense potential in the discovery of novel bioactive compounds [**5**].

Actinomycetes or Actinobacteria are a group of aerobic, free living, frequently filamentous, branched, unicellular Gram positive bacteria with DNA with high percentage GC (55-75%) in DNA **[6, 7]**. They are ubiquitously prevalent diverse groups of prokaryotic microorganisms, which are known to synthesize new secondary metabolites that show a wide range of biological activities such as antibacterial, antifungal, anticancer, cyto-toxic, anti-inflammatory, anti-parasitic, antiviral, antioxidant, anti-HIV, neurological and immunosuppressive agents **[8, 9]**.

As for novel bioactive commpounds, actinobacteria produce approximately two-thirds of all known antibiotics, the majority of which 75% - 80% have been obtained from the genus *Streptomyces* **[10-13]**. *Streptomyces* has the ability to produce clinically important bioactive compounds and is the most productive genus in the microbial World **[14, 15]**.

In recent years multi-drug resistant pathogens has emerged due to extensive use of antimicrobials in medicine, animal husbandry and agriculture **[16]**. Study of such kind of economically, industrially viable actinomycetes more particularly *Streptomyces* could offer an insight into the formulation of new antibiotics against the drug resistant pathogens **[17]**.

As the mangrove ecosystem is largely unexploited source for new antibiotics, isolation of actinomycetes from such unique unexplored natural habitats will provide rare bio active metabolites. Therefore, exploration of unexplored ecosystems for actinomycetes is necessary for the identification of novel antimicrobial metabolites. The objective of present study is isolation and screening of actinomycetes from mangrove sediment samples (Machilipatnam) for potential bioactive compounds.

METHODS AND MATERIALS

Sampling and pre-treatment of soil

Soil samples were randomly collected from different locations of mangrove ecosystem of Machilipatnam, South coast of Andhra Pradesh, India. Samples were collected from 6 cm to 10 cm depth, and brought to the laboratory and air dried at room temperature. The samples were pretreated with calcium carbonate (10:1 w/w) and incubated at 37°C for 4 days **[18]**.

Isolation of Actinobacteria

The pretreated soil samples were serially diluted in sterile distilled water and plated on selective medium such as Yeast extract malt extract dextrose (YMD) agar supplemented with 3% NaCl **(19)**. The medium was adjusted to pH 7.0 and 0.1 mL of diluted sample was spread over YMD agar supplemented with nystatin (25 µg/mL) and streptomycin (25 µg/mL) to both reduce the fungal and bacterial contamination respectively and incubated at 30±2°C for three weeks. Actinobacterial colonies were picked up, sub cultured and preserved on YMD agar slants at 4°C **[20-22]**. Among the 10 strains screened for antimicrobial compounds, the strain designated as VLCH-1 was found potent.

Identification of the potent strain VLCH-1 by polyphasic taxonomy:

Morphological, Cultural, Physiological and biochemical characteristics of the strain

The potent actinobacterial strain VLCH-1 was characterized by cultural, morphological, physiological, biochemical and molecular methods. The nature of mycelium and spore arrangement of the strain was observed by slide culture method under a compound microscope (model Motic- BA410) **[23]**.The morphological characteristics were studied using scanning electron microscopy (SEM: Model- JOELJSM 5600, Japan) of 4-day old culture grown on YMD agar medium. The strain was grown on six International Streptomyces Project (ISP) media and four non-ISP media to observe the cultural characteristics such as color of aerial mycelium, substrate mycelium, pigment production and spore formation **[24]**. Melanin pigment production was observed by culturing the strain on YMD (ISP-2) medium **[25]**. Hydrolysis of starch, nitrate reduction and H_2S production were also evaluated **[26, 27]**. Physiological characteristics such as influence of pH (5-9), temperature (20-45°C) and effect of NaCl on growth of strain were analyzed. The sensitivity of the strain to different antibiotics was also determined by paper disc method **[28]**.

Molecular identification

The genomic DNA used for the polymerase chain reaction (PCR) was prepared from the colonies grown on YMD agar for 3 days. The total genomic DNA extracted from the isolate was isolated by employing the DNA purification Kit (Pure Fast® Bacterial Genomic DNA purification kit, Helini Bio molecules, India) according to the manufacturer protocol. Conditions of the PCR were standardized with initial denaturation at 94°C for 3 minutes followed by 30 cycles of amplification (Denaturation at 94°C for 60 seconds, annealing temperature of 55°C for 60 seconds, and extension at 72°C for 60 seconds and an addition of 5 minutes at 72°C as final extension).The amplification reactions were carried out with a total volume of 50µL in a gradient PCR (Eppendorf, Germany). Each reaction mixture contained 1 µL of DNA, 1 µL of 10 *P* mol forward 16S actino specific primer (5'AAATGGAGGAAGGTGGGGAT-'3), 1 µL of 10 *P* mol reverse 16S actino specific primer (5'- AGGAGGTGATCCAACCGCA-'3), 25 µL of master mix, and 22 µL of molecular grade nuclease free water. The separation was carried out at 90 Volts for 40 minutes in TAE buffer with 5 µL of ethidium bromide. PCR product was analyzed using agarose gel (1%) and the fragment was purified (Helini Pure Fast PCR clean up kit, Helini Bio molecules, India) as per the manufacturer's instructions. The bands were analyzed under UV light and documented using Gel Doc. The direct sequencing of PCR products was performed by dideoxy chain termination method using 3100-Avant genetic analyzer (Applied Biosystems, USA).

Pair wise sequence alignment

The gene sequence of the isolate VLCH-1 was aligned using BLAST against the gene library available for *Streptomyces* species in the NCBI and the GenBank. Pairwise evolutionary distances were computed by MEGA-6 software.

Multiple sequence alignment

The phylogenetic analysis was conducted using the maximum parsimony method of the isolate using BLAST and CLUSTAL W. The closely related homologous isolates were identified, retrieved and compared to the sequence of the isolated strains using CLUSTAL W available with the MEGA 6 Version [**29**].

Nucleotide sequence accession numbers

The 16S rRNA gene sequence of the isolate VLCH-1 was registered in the GenBank database.

Growth pattern and antimicrobial potential of VLCH-1

To determine growth pattern, the isolate was inoculated into 250 ml flasks containing 100 ml YMD broth and incubated at 30±2°C on a rotary shaker at 180 rpm. The flasks were harvested at 24 h interval and growth of the isolate was determined by taking dry weight of biomass. The culture filtrate obtained after separating the biomass was extracted with ethyl acetate. The ethyl acetate extract was evaporated to obtain crude metabolite which was used to determine antimicrobial activity.

The antimicrobial activity of the solvent extract was determined by agar well diffusion method [**30**]. Ethyl acetate itself was used as negative control. The wells were made by using sterile cork borer (5 mm diameter). The test bacterial cultures were sub cultured on nutrient agar medium and incubated at 37 °C for 24 h and test fungal cultures were sub cultured on Sabouraud dextrose agar medium at 35 °C for 48 h. 80 µl of the crude extract and 80 µl of negative control were poured in to separate wells. The standard antibiotic disc was placed on agar surface as positive control. The plates were incubated at 37°C for 48 h and inhibition zones (mm) were measured after 24-48 h **[31, 32]**.

Test organisms

Bacteria: *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (ATCC 6633), *Bacillus megaterium* (NCIM 2187), *Xanthomonas campestris* (MTCC 2286), *Proteus vulgaris* (ATCC 6380), *Pseudomonas aeruginosa* (ATCC 9027) and *Escherichia coli* (ATCC 9027).

Fungi: *Aspergillus flavus* (ATCC 189), *Candida albicans* (MTCC 183) and *Penicillium citrinum* (MTCC 6849).

RESULTS AND DISCUSSION

A total of 10 actinobacterial strains were isolated from the mangrove habitats of Machilipatnam and designated as VLCH-1 to VLCH-10. Among them, one actinobacterial strain VLCH-1was found to be potent and exhibited strong antimicrobial activity against test bacteria and fungi.

Cultural characteristics of the strain VLCH-1

The cultural characteristics of the strain are represented in **Table 1**. The strain VLCH-1 exhibited good growth on tryptone yeast extract-agar, YMD agar, starch casein agar and humic acid vitamin-B agar media while the growth was moderate on tyrosine agar, nutrient agar and oat meal agar media. The growth was not found on inorganic salts starch agar, glycerol aspargine agar and Czapek – dox agar media. The color of aerial mycelium was gray and substrate mycelium was pale yellow on different media. Soluble black colored pigment production was observed on all the media tested except nutrient agar.

Morphological characteristics of the strain VLCH-1

Micro-morphological view of the strain was examined under Scanning electron microscopy. The strain displayed massive sporulation with branched mycelium and abundant aerial hyphae. The Spores of the strain was spherical, globose with smooth-surface. Paired spores on hyphae were observed **(Figure 1)**.

Biochemical and Physiological characteristics of the strain VLCH-1

The physiological and biochemical characteristics were significant tools for identification of actinobacteria. The strain VLCH-1 exhibited a positive response to indole production, urease activity, gelatin liquefaction, methyl red and catalase activity but negative to H_2S production, Voges-Proskauer and citrate utilization. The strain VLCH-1 could also produce enzymes like L-asparaginase, amylase and glutaminase **[23-25] (Table 2).**

The strain showed good growth at pH range between 5 and 9 with the optimum being 7 and the range of temperature for growth was 30-40°C with the optimum being 35°C. Sodium chloride tolerance of the strain was also studied as the salt concentration has a profound effect on the production of antibiotics. The strain could grow well in the medium supplemented with 3% sodium chloride and showed tolerance up to 7%.

Though the strain utilized a wide range of carbon sources, maltose, D-glucose and sorbitol supported good growth of the strain. The strain showed sensitivity to Gentamicin, imipenem, vancomycin, ampicillin, chloramphenicol and tetracycline.

Molecular characterization of the potent strain VLCH-1:

Based on the morphological, cultural, physiological, biochemical and molecular characteristics, the strain has been included under the genus *Streptomyces* and deposited at NCBI genbank with an accession number **MZ951165.** The partial sequence was aligned and compared with all the 16S rRNA gene sequence available in the GenBank database by using the multi sequence advanced BLAST comparison tool that is available in the website of NCBI. The phylogenetic analysis of the 16S rRNA gene sequence was aligned using the CLUSTAL W program from the MEGA 6 Version **(29).** Phylogenetic tree was constructed using MEGA software Version 6 using maximum parsimony method **(Figure 3)**.

Growth pattern and antimicrobial profile of the strain *Streptomyces cinnamonensis* - VLCH-1:

The growth curve and antimicrobial profile of *S. cinnamonensis* VLCH-1 were studied at regular intervals up to 8 days in batch culture. The stationary phase of the strain extended from 144 h to 168 h of incubation **(Figure 5).** The bioactive secondary metabolites obtained from 6-day-old culture showed high antimicrobial activity against the test microbes.

The secondary metabolites obtained from 5-day-old culture extracts of *S. albogriseolous* VJMS-7 **[33],** *S. violaceoruber* VLK-4 **[34]** and *S. cellulosae* VJDS-7 **[35]** exhibited high antagonistic activity. Similarly, the antimicrobial metabolites extracted from 6-day-old culture extracts of *S. vinaceusdrappus* VJMS-4, *S. rectiverticillatus* VJMS-8 **[36]** and 4-day-old culture extracts of *S. cheonanensis* VUK – A, *S. tendae* TK-VL 333 **[37, 38]** showed high antimicrobial activity against the test microorganisms.

The antimicrobial spectrum of the strain cultured on YMD broth for 8 days was tabulated **(Table 5)**. The metabolites extracted from 6-day-old culture showed maximum activity against *B. megaterium*, *E. coli* and *K. pneumoniae*. In case of fungi, *C. albicans* showed high sensitivity.

Statistical Analysis: Readings were taken as the mean ± standard deviation of the mean of three replicates calculated using Microsoft Excel XP 2007.

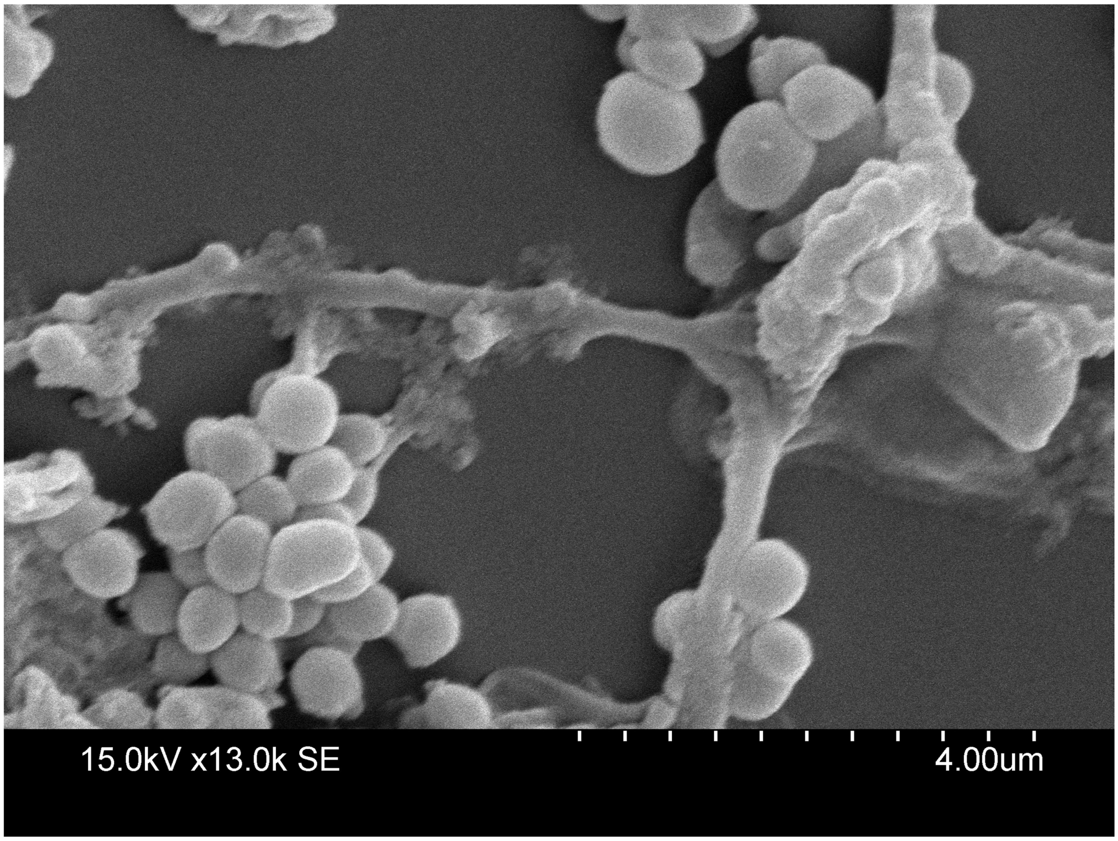

Figure 1: Scanning Electron Microscopic Photograph of *Streptomyces cinnamonensis* VLCH-1

Table 1: Cultural characteristics of the strain VLCH-1

Medium	Growth	A.M	S.M	Pigmentation
Tryptone yeast extract agar (ISP-1)	Good	Gray	Pale yellow	Black
Yeast extract malt extract dextrose agar (ISP-2)	Good	Gray	Pale yellow	Black
Oat-meal agar (ISP-3)	Moderate	Gray	Pale yellow	Black
Inorganic salts starch agar (ISP-4)	-	-	-	-
Glycerol asparagine agar (ISP-5)	-	-	-	-
Tyrosine agar (ISP-7)	Moderate	Gray	Pale yellow	Black
Starch-casein agar	Good	Gray	Pale yellow	Black
Humic acid vitamin-B agar	Good	Gray	Pale yellow	Black
Czapek-Dox agar	-	-	-	-
Nutrient agar	Moderate	Gray	Pale yellow	No

***AM- Aerial mycelium, *SM- Substrate mycelium. -: No growth, *ISP: International Streptomyces Project**

Table 2: Morphological, Biochemical and Physiological characteristics of VLCH-1

Character	Response
Morphological characters	
Sporophore morphology	Recti flexible
Color of aerial mycelium	Gray
Color of substrate mycelium	Pale yellow
Physiological characters	
Grams reaction	+
Production of melanin pigment	+
Range of temperature for growth	30 – 40 ^{o}C
Optimum temperature for growth	35 ^{o}C
Range of pH for growth	5 – 9
Optimum pH for growth	7
NaCl tolerance	Up to 7%
Optimum NaCl Concentration	3%
Biochemical characters	
Catalase production	+
Urease production	+
H_2 S production test	-
Nitrate reduction	+
Starch hydrolysis	-
Gelatin liquefaction	+
Methyl red test	+
Voges proskauer test	-
Indole production	+
Citrate utilization	-
Enzymatic activity	
Amylase	P
Cellulase	N
Asparaginase	P
Glutaminase	P
Protease	N

Table 3: Utilization of the carbon sources by the strain VLCH-1 (w/v)*

Carbon Sources	Response
D-glucose	+++
Maltose	+++
Cellulose	-
Sucrose	+
Lactose	+
Sorbitol	+++
Dulcitol	+
Fructose	+
Galactose	++
Starch	+
Mannitol	+

* Growth of the strain measured as dry weight of the mycelium '+++'-good growth; '++'-moderate growth; '+'-weak growth; '-'indicates negative/no growth

Table 4: Antibiotic susceptibility/resistance of VLCH-1

Antibiotics (µg/disc)	Susceptibility/Resistance
Gentamicin (10µg)	S
Vancomycin (30 µg)	S
Tetracycline (30 µg)	S
Penicillin (10 µg)	R
Chloramphenicol (50 µg)	S
Clindamycin (25 µg)	R
Imipenem (10 µg)	S
Cefixime (30 µg)	R
Ampicillin (10 µg)	S
Amikacin (10µg)	R

*S – Sensitive ; *R - Resistant

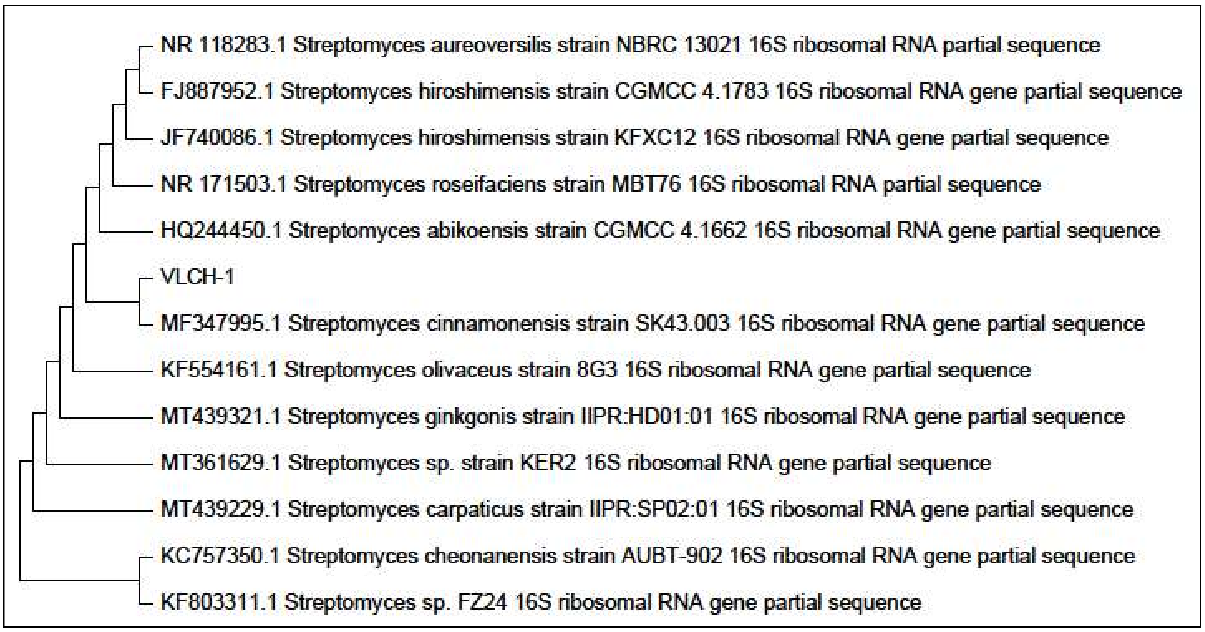

Figure 3: Maximum parsimony tree based on partial 16S rRNA gene sequence showing relationship between strain VLCH-1 and related members of the genus *Streptomyces*

Table 5: Antimicrobial activity of *Streptomyces cinnamonensis* - VLCH-1

Test organism name	Zone of Inhibition (mm)
Bacteria	**VLCH-1**
Staphylococcus aureus	20
Escherichia coli	24
Xanthomonas campestris	20
Pseudomonas aeruginosa	17
Bacillus megaterium	22
Streptococcus mutans	20
Klebsiella pneumoniae	23
Fungi	
Candida albicans	22
Aspergillus flavus	12
Penicillium citrinum	14

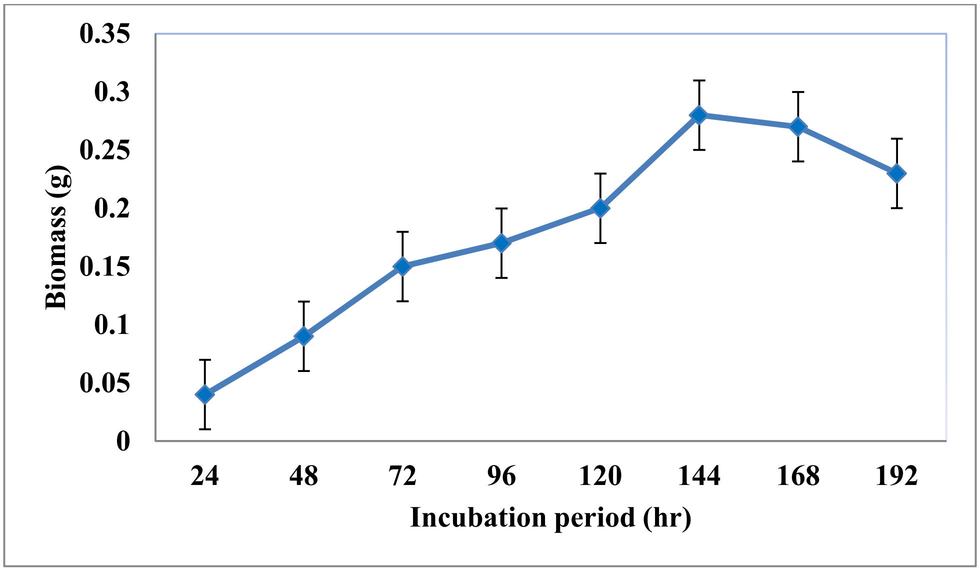

Figure 5: Growth pattern of the strain *Streptomyces cinnamonensis* - VLCH-1

CONCLUSION

The present investigation highlights the antimicrobial potential of *Streptomyces cinnamonensis* VLCH-1. It is evident from the present study that mangrove habitats of Machilipatnam, serve as a good source for the isolation of potent actinomycetes with broad spectrum antimicrobial activity. Further study on chemical characterization of bioactive compounds of the isolate is in progress.

ACKNOWLEDGEMENT

The authors are thankful to UGC-BSR (MVL) and UGC-SAP (ChCh) for providing the financial support to carry out the research work and also to the Department of Botany and Microbiology for providing the laboratory facilities.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

REFERENCES:

[1] Palla, M.S., Guntuku, G.S., Muthyala, M.K.K., Pingali, S. and Sahu, P.K., 2018. Isolation and molecular characterization of antifungal metabolite producing actinomycete from mangrove soil. *Beni-Suef University Journal of Basic and Applied Sciences*, *7*(2), pp.250-256.

[2] Simoes, M.F., Antunes, A., Ottoni, C.A., Amini, M.S., Alam, I., Alzubaidy, H., Mokhtar, N.A., Archer, J.A. and Bajic, V.B., 2015. Soil and rhizosphere associated fungi in gray mangroves (Avicennia marina) from the Red Sea—a metagenomic approach. *Genomics,* *proteomics & bioinformatics*, *13*(5), pp.310-320.

[3] Thatoi, H., Behera, B.C., Mishra, R.R. and Dutta, S.K., 2013. Biodiversity and biotechnological potential of microorganisms from mangrove ecosystems: a review. *Annals of Microbiology*, *63*(1), pp.1-19.

[4] Amrita, K., Nitin, J. and Devi, C.S., 2012. Novel bioactive compounds from mangrove derived actinomycetes.

[5] Singh, V., Haque, S., Singh, H., et al., 2016. Isolation, Screening, and Identification of Novel Isolates of Actinomycetes from India for Antimicrobial Applications. Front. Microbiol. 7, 1921.

[6] Radhakrishnan, M., Suganya, S., Balagurunathan, R. and Kumar, V., 2010. Preliminary screening for antibacterial and antimycobacterial activity of actinomycetes from less explored ecosystems. *World Journal of Microbiology and Biotechnology*, *26*(3), pp.561-566.

[7] Naragani, K., Munaganti, R.K., Sirigiri, C.K. and Muvva, V., 2014. Antimicrobial potential of Streptomyces violaceoruber VLK-4 isolated from South coast of Andhra Pradesh, India. *Int J Pharm Sci Rev Res*, *25*, pp.125-29.

[8] Dharmaraj, S., 2010. Marine *Streptomyces* as a novel source of bioactive substances. *World Journal of Microbiology and Biotechnology*, *26*(12), pp.2123-2139.

[9] Valli, S., Suvathi, S.S., Aysha, O.S., Nirmala, P., Vinoth, K.P. and Reena, A., 2012. Antimicrobial potential of Actinomycetes species isolated from marine environment. *Asian Pacific Journal of Tropical Biomedicine*, *2*(6), pp.469-473.

[10] Lu, Q.P., Ye, J.J., Huang, Y.M., Liu, D., Liu, L.F., Dong, K., Razumova, E.A., Osterman, I.A., Sergiev, P.V., Dontsova, O.A. and Jia, S.H., 2019. Exploitation of potentially new antibiotics from mangrove actinobacteria in maowei sea by combination of multiple discovery strategies. *Antibiotics*, *8*(4), p.236.

[11] Sengupta, S., Pramanik, A., Ghosh, A. and Bhattacharyya, M., 2015. Antimicrobial activities of actinomycetes isolated from unexplored regions of Sundarbans mangrove ecosystem. *BMC microbiology*, *15*(1), pp.1-16.

[12] Saritha, M., Arora, A., Singh, S. and Nain, L., 2013. *Streptomyces griseorubens* mediated delignification of paddy straw for improved enzymatic saccharification yields. *Bioresource technology*, *135*, pp.12-17.

[13] Kavitha, A., Vijayalakshmi, M., 2013. Evaluation of bioactive compounds produced by *Nocardia levis* MK-VL113 & *Streptomyces tendae* TK-VL333 for cytotoxic activity. Indian J. Med. Res. 137, 391–393.

[14] R. Usha, P. Anathasevi, C.K. Venil and M. Palaniswamy: Antimicrobial and Antiangiogenesis Activity of Streptomyces paravulus KUAP106 from Mangrove soil. European J of Biological science 2012; 4(4):77-83

[15] Hong, K., 2013. Actinomycetes from mangrove and their secondary metabolites. *Wei sheng wu xue bao= Acta microbiologica Sinica*, *53*(11), pp.1131-1141.

[16] Hamedi, J., Imanparast, S. and Mohammadipanah, F., 2015. Molecular, chemical and biological screening of soil actinomycete isolates in seeking bioactive peptide metabolites. *Iranian journal of microbiology*, *7*(1), p.23.

[17] Guodong chen, Hao gao, Jinshan tang, Yuanfan huang, Ying chen, Ying wang, Huinan zhao, Haipeng lin, Qingyi xie, Kui hong, Jia li, and Xinsheng yao: Benzamides and Quinazolines from a Mangrove Actinomycetes *Streptomyces* sp. (No. 061316) and their inhibiting Caspase-3 catalytic activity *in vitro.*Chem. Pharm. Bull 2011: 58(4): 447-451

[18] Suresh, R.S., Younis, E.M. and Fredimoses, M., 2020. Isolation and molecular characterization of novel Streptomyces sp. ACT2 from marine mangrove sediments with antidermatophytic potentials. *Journal of King Saud University-Science*, *32*(3), pp.1902-1909.

[19] Ragalatha R., Vijayalakshmi M., Mallikarjuna K., and Veer Kishore I., Bioactivity and taxonomic studies of *Nocardiopsis dassonvillei* and Nocardiopsis species from Marine sediments of Bay of bengal, India. *Int J Pharm Sci. and Resea.2020; 11(4): 1790-1801.*

[20] Bizuye, A., Moges, F. and Andualem, B., 2013. Isolation and screening of antibiotic producing actinomycetes from soils in Gondar town, North West Ethiopia. *Asian Pacific journal of tropical disease*, *3*(5), pp.375-381.

[21] Kavitha A., Vijayalakshmi M., Sudhakar P., Narasimha G., Screening of actinomycete strains for the production of antifungal metabolites. *Afr JMicrobiol Res*, 4:27-32, (2010).

[22] Jinhua Z: Improvement of an isolation medium for actinomycetes. Modern Appl Sci 2011; 5: 124-27.

[23] Hayakawa M., Studies on the isolation and distribution of rare actinomycetes in soil. *Actinomycetologica*, 22:12-9, (2014).

[24] Yasmeen S, Vijayalakshmi M and Rajesh Kumar M: Taxonomic characterization of rare actinobacteria isolated from mangrove ecosystem. Int J Curr Research 2016; 8(05): 31060-31066.

[25] Bizuye A, Moges F and Andualem B: Isolation and screening of antibiotic-producing actinomycetes from soils in Gondar town, North West Ethiopia. Asian Pac J Trop Dis 2013; 3(5): 375- 81.

[26] Kavitha A., Vijayalakshmi M., Sudhakar P., Narasimha G., Screening of actinomycete strains for the production of antifungal metabolites. *Afr JMicrobiol Res*, 4:27-32, (2010).

[27] Naradala PR, Vijayalakshmi M, Mangamuri UK and Kavikishore PB: Exploration of potent actinobacteria *Nocardiopsis halotolerans* VJPR-2 isolated from Mangrove habitats. Asian J Pharm Clin Res 2016; s113-117.

[28] H. Arifuzzaman, M. R. Khatun and H. Rahman: Isolation and screening of actinomycetes from Sundarbans soil for antimicrobial activity. African J of Biotechnology 2012; 9(29): 4615-4619.

[29] Tamura K, Stecher G, Peterson D, Filipski A and Kumar S: MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 2013; 30(12): 2725-29.

[30] Kapur, M.K., Solanki, R., Das, P., Kumar, M. and Kumar, P., 2018. Antimicrobial activity analysis of bioactive compounds from soil

actinomycetes. *J Pharm Chem Biol Sci*, *6*, pp.178-187.

[31] M. Kavya Deepthi I, M. Solomon sudhakar and M. Nagalakshmi Devamma: Isolation and screening of streptomyces sp. From coring mangrove soils for enzyme production and antimicrobial activity. International J of Pharmaceutical, chemical and biological sciences 2012; 2(1): 110-116.

[32] Mogili, M.S. and Muvva, V., selective isolation and characterization of *nocardiopsis flavescens* vjms-18 from coastal regions of Andhra Pradesh, India. Int J Pharm Res 2019; 10(8): 1000-08.

[33] Mary swapna mogili, Vijayalakshmi muvva. Antimicrobial potential of *Streptomyces albogriseolus* VJMS -7 isolated from coastal regions of Guntur district, Andhra Pradesh, India. Asian jr. of microbial.biotech.env.sc.21:2019:688-694.

[34] Naragani K., Rajesh Kumar M., Vijayalakshmi M., Antimicrobial potential of *Streptomyces violaceoruber* VLK-4 isolated from Southcoast of Andhra Pradesh, India. *Int J Pharm Sci Rev Res*, 25:125-9, (2014).

[35] Mani deepa, I. Vijayalakshmi, M. and Rajesh kumar, M. 2015. *Streptomyces cellulosae* VJDS-1, a promising source for potential bioactive compounds. *Int J Pharm and Pharmaceutical Sci*. 7 (7).

[36] Mogili, M.S. and Muvva, V., isolation, identification and antimicrobial potential of marine Streptomyces spp from coastal regions of Andhra Pradesh, India. Int J Pharm Biol Sci 2018; 8(3):2230-7605

[37] Usha Kiranmayi, M. Sudhakar, P. Krishna, N. and Vijayalakshmi, M. 2012. Influence of cultural conditions for improved production of bioactive metabolites by *Streptomyces cheonanensis* VUK-A Isolated from coringa mangrove ecosystem. Curr Trends Biotechnol Pharm. 6:99-111.

[38] Kavitha, A. and Vijayalakshmi, M. 2010. Optimization and purification of L-asparaginase produced by *Streptomyces tendae* TK-VL_ 333. Z Naturforsch. 65:528-31.