Advances in Bioresearch
Adv. Biores., Vol 15 (2) March 2024: 438-445

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.15.2.438445

Advances in Bioresearch

ORIGINAL ARTICLE

Antimicrobial activity of actinomycetes isolated from mangrove rhizosphere soil in Vellar estuary along South East Coast of India

R. Kiruthiga[1]., G. Thiruneelakandan.[2*]
1,2. Marudhupandyar Arts and Science college, Thanjavur
* Corresponding Author: R. Kiruthiga, Research Scholar, Marudhupandiyar College, Thanjavur. Affiliated to Bharathidasan University, Trichy.
E mail: kiruthigaoppila@gmail.com

ABSTRACT
Marine actinomycetes are commonly used to manufacture new medicinal compounds due to their enormous diversity, however, this is currently under investigation. In this study, we identified five marine actinomycetes from the mangrove rhizosphere soil of the Vellar estuary in Cuddalore, Tamil Nadu, India (VM 01, VM 02, VM 03, VM 04,). The isolates' morphological and physiochemical characteristics were identified. Based on the morphological and physiochemical properties 70.8 % of isolates were identified as a Streptomyces spp., and 45.3% as a Saccharopolyspora spp. Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 25922), Klebsiella pneumoniae (ATCC 700603), and Pseudomonas aeruginosa (ATCC 27853) were used as test organisms for primary screening for antimicrobial activity, and secondary screening was done using the agar well diffusion method with ethyl acetate as the solvent for extraction. All the isolates VM04, VM 03, and VM01 were found to have antimicrobial activity with maximal zones of inhibition of 16mm, 9mm, and 9mm, respectively, at a concentration of 10 mg /mL against E. coli. The isolates shown negative results against other selected pathogens. The outcome of these findings may be crucial for future investigations towards the invention of wide-ranging antibiotics for therapeutic applications.
KEYWORDS: *Actinomycetes, pigments, antimicrobial activity*

Received 21.02.2024 Revised 01.03.2024 Accepted 11.03.2024

How to cite this article:
R. Kiruthiga.G. Thiruneelakandan Antimicrobial activity of actinomycetes isolated from mangrove rhizosphere soil in Vellar estuary along South East Coast of India. Adv. Biores., Vol 15 (2) March 2024: 438-445.

INTRODUCTION

Mangroves, which grow in intertidal zones along tropical and subtropical coasts, are one of the world's most abundant ecosystems. The mangrove environment provides a rich supply of unique plants, bacteria, algae, and animals for the creation of biologically active compounds [1]. According to [2], the environments are defined by sandy or muddy soil with high salinity, a rather wide range, a high average temperature, and strong winds, mangrove soils create significant environments for the development of various microorganisms. The most prevalent microorganisms in mangroves are bacteria and fungi, which support the productivity of ecosystems by engaging them in important nutrient cycles [3]. Additionally, such bacteria can create bioactive metabolites that may be used for both industrial and therapeutic applications. Mangrove soil isolated fungi have been shown to generate medicinal substances including antimicrobials antiviral, antioxidants, and anticancer as well as industrial enzymes like lipase, cellulase, protease, and pectinase [4]. Actinomycetes are one of the major bacterial species that produce bioactive chemicals in mangrove soils. According to [5], actinomycetes are filamentous Gram-positive bacteria with morphology resembling fungi, a large genome of more than 8 Mbp, high G+C content, and biosynthetic gene clusters relevant to the production of secondary compounds. Actinomycetes that have been identified from mangrove sediments and soils have the potential to produce antibiotics, anticancer drugs, and antioxidants. Actinomycetes are known to be the primary manufacturers of antibiotics. Actinomycetes manufacture more than 80% of the antibiotics that are now in use, including tetracycline, macrolide, chloramphenicol, nucleosides, and polyenes [6], [7], [8]. Streptomyces species in particular are known to be the largest producers of antibiotics among them [9]. In the current investigation, soil

samples from the mangrove rhizosphere, which were obtained from several areas of the cellar estuary, were isolated and screened for actinomycetes that produce antimicrobials. To analyze and report the distribution of the antimicrobial activity of microorganisms gathered from sample locations, the present investigation is important.

MATERIAL AND METHODS

Sample collection

Using the purposive sampling technique, soil samples were taken from the Vellar estuary Mangrove, Parangipettai, and Cuddalore district at two separate places. Location I consisted of 10 sample points, while Location II consisted of 8 sample points. We collected 100 g of dirt from each sampling location. Using a soil boring, samples were obtained from 0 to 15 cm deep [10], and they were then placed in sterile plastic bags. The obtained sample was examined for physicochemical characteristics, such as soil pH (4–6.4), salinity (0–15%), temperature (27–28°C), humidity (70–82%), and temperature (27–28°C)[11].

Soil sample preparation

For a week, soil samples were heated and dried to get rid of any undesired Gram-negative bacteria. samples that have been baked in an oven for 30 minutes at 65°C after being air-dried at ambient temperature, placed in an aluminum cup, then heated for another 30 minutes[12], [13].

Isolation of Actinomycetes

The soil samples were inoculated into Starch Casein Agar (SCA) medium supplemented with 0.1% chloramphenicol and 0.1% griseofulvin using the pour plate technique [[14]]. The soil samples were suspended in sterile distilled water (1:9 w/v). After that, the samples were kept at 27°C for 1-4 weeks [12].

Characterization and identification

The actinomycetes were identified using macroscopic and microscopic examinations, as well as physiological tests, as recommended by Bergey's Manual of Systematic Bacteriology, 2nd Edition, Vol 5, Actinomycetes, Part A. Macroscopic Characterization. Aerial mycelium, submerged mycelium, color, and diffusible pigments were all detected in isolated actinomycetes. Microscopic inspection was carried out using a cover slip and the Gram-staining technique. The solidified starch casein agar medium plate had a sterile coverslip put into it at a 45° angle to the agar surface. Along the medium's surface where it meets the surface of the submerged coverslip, an actinomycete isolate was injected. For four days, it was incubated at 28 °C. Using sterile forceps, the cover slip was removed and put on a unique, clean glass slide, where it was then viewed using an oil immersion objective.[15]. For each isolate, a loopful of a pure culture colony that had been incubating for about 7 days was placed in starch-casein broth and incubated at 28°C for 4 days. The culture suspension was employed for various protein consumption tests, catalase, and oxidase tests, and sugar utilization tests after the emergence of turbidity. A variety of hydrolysis tests were conducted, including those for gelatine, casein, lipids, and starch.

Antimicrobial activity test

Two actinomycetes isolates were chosen for antibacterial activity testing. The isolates were cultured for one week at 32°C in Nutrient Broth (NB medium). Crude antimicrobial compounds (CACs) were extracted from supernatants by centrifugation at 6000 rpm for 10 minutes. The disc diffusion technique was used to conduct an antibacterial test against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), and *Pseudomonas aeruginosa* (ATCC 27853).

RESULTS

Sampling sites

The Mangrove Centre Vellar estuary is an artificial conservative zone. *Rhizophora* and *Avicennia* species dominate the vegetation. Soil samples were taken from locations I (105 m from the sea) and II (40 m from the sea) for this investigation. (Table 1) shows the soil qualities that were sampled. Locations I and II were split into I-A and II-A, which have pH ranges of 5 to 6, and I-B and II-B, which have pH ranges of 4 to 8, which are relatively acidic.

Isolation of actinomycetes from mangrove Rhizosphere soil

There were four isolates of actinomycetes found at the sample site of location I, whereas none were found at location II (Table 2). It was discovered specifically by the VM.2 isolate that actinomycetes could flourish in acidic soil. The four isolates differ in a variety of macroscopic and microscopic ways (Table 3). Figure 1 depicts the isolates' colony morphology, and Figure 2 displays the Gram-staining results. Different colony morphological characteristics may be seen in four isolates of actinomycetes (Table 3). Actinomycetes colonies often have microscopic features such as being tiny, dry, wrinkled, fibroma-like, or

velvety on the surface. The VM04 isolation in this investigation was dry, the VM 03 isolate had a fibrotic surface, the VM02 isolate had a minor wrinkle, the VM 01 isolate had a smooth surface, and the VM 01 isolate also had a silky texture. Furthermore, VM 02 had grey aerial mycelium, VM 04 had white aerial mycelium, VM 01 had white aerial mycelium with filaments, and VM 03 had yellow aerial mycelium with scraper edges.

Screening of antimicrobial activity

Actinomycetes isolated from Vellar estuary mangrove soil may inhibit the development of *E. coli* ATCC 25922, but not of *S. aureus* ATCC 25923 and *Klebsiella pneumoniae* (ATCC 700603) (Table 4). It might be related to the isolates' capacity to limit the development of Gram-negative bacteria, specifically E. coli shown in figure 3. The VM04 isolate has the highest inhibition zone against *E. coli* ATCC 25922 (16.65 ± 2.33 mm), whereas the VM 02 isolate has the smallest (8.77 ± 0.42 mm). The size of the clear zone in the initial screening test findings suggested that VM 04 had the greatest inhibitory zone. VM 04's inhibitory zone was compared to the negative control and chloramphenicol and amoxicillin as positive controls (Figure 4).

Identification of the isolate with the greatest antibacterial activity

Because the VM 04 isolate had the largest inhibitory zone against *E. coli*, it was studied further to discover its genus. Table 6 shows the results of the biochemical analysis on the VM 04 isolate. LIB.2 is a Gram-positive rod bacterium with a grey aerial mycelium and a spiral-shaped spore chain that produces catalase. The LIB.2 isolates shared 92.8% of its physiological features with the Streptomyces genus [16].

Table 1. Soil physiochemical parameters

Location	pH	Temp. (°C)	Salinity (%)	Moisture
LI -a	6.3 ± 0.2	27.6 ± 0.4	9.1 ± 5.2	7.5 ± 0.5
LI -b	4.1 ± 0.1	28.0 ± 0.1	15.0 ± 0.2	8.2 ±0.5
LII -a	5.8 ±0.4	28.7 ± 0.4	0.2 ± 0.0	8.1 ± 0.2
LII -b	4.7 ± 0.0	29.1 ± 0.2	8.2 ± 0.2	8.3 ± 0.1

Table 2. Actinomycetes isolated from mangrove rhizosphere soil in Vellar estuary

Location	Code of the sample	Amount of the sample
LI -a	VM 01	2
LI -b	VM 02	2
LII -a	VM03	2
LII -b	VM 04	2

Table 3. Characteristics of Actinomycetes isolates from mangrove rhizosphere soil in Vellar estuary

		Macroscopic			Microscopic	
Code	**Color of aerial mycelium**	**The color of the substrate mycelium**	**Change the color of the medium**	**Gram**	**Spore**	**The shape of the spore chain**
VM 01	White	Gray	No	+(purple)	Yes	*Streptomyces*/Spiral
VM 02	Gray	Pink	Pink	+(purple)	Yes	*Streptomyces*/Straight
VM03	White	Blackish Green	No	+(purple)	Yes	*Saccharopolyspora* spp
VM 04	Yellow	Yellow	Yellow	+(purple)	Yes	*Saccharopolyspora* spp

Table4. The inhibitory zone of Actinomycetes against *Escherichia coli, Staphylococcus aureus,* and *Klebsiella pneumonia*

Code of isolate	Diameter of the inhibitory zone (mm)		
	***E. coli* ATCC 25922**	***S. aureus* ATCC 25923**	***K .pneumoniae* ATCC 700603**
VM 04	16.65 ± 2.33	0	0
VM03	9.78±0.46	0	0
VM01	9.25±2,56	0	0
VM 02	8.77 ± 0.42	0	0

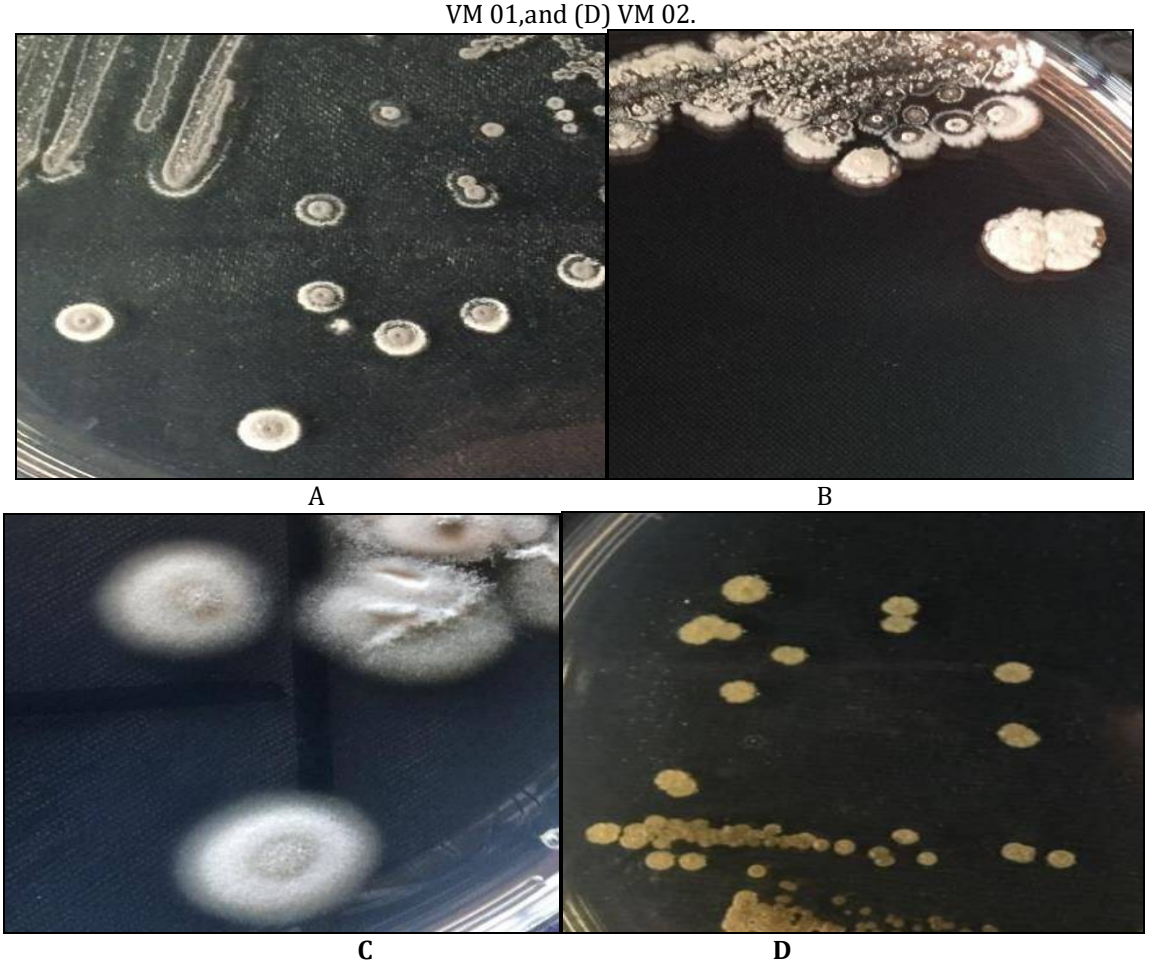
Table 5. The inhibitory zone of VM 04 and positive control against *Escherichia coli* ATCC 25922

Sample	The average diameter of the Inhibitory zone (mm)
VM 04	11.58±1.85
Chloramphenicol0,01%	15.45±0.04
Amoxicillin0,01%	26.89±0.59

Table 6. Biochemical features of the isolate of VM 04

Tests	Results
Gram	+
Color of aerial mycelium	Gray
The shape of the spore chain	Spiral
Catalase	+
Motility	-
Glucose	-
Xylose	-
Arabinose	-
Rhamnose	-
Raffinose	-
Mannitol	-
Inositol	-
Sucrose	-

Figure 1. Macroscopic characteristics of actinomycetes isolated from location I. (A) VM 04, (B) VM03,(C) VM 01,and (D) VM 02.



A B

C D

Figure 2. Microscopic characteristics of actinomycetes isolated from mangrove soil obtainedin Vellar estuary by Gram staining. The arrow shows the spore chain.(A) VM 04, (B) VM03,(C) VM 01,and (D) VM 02

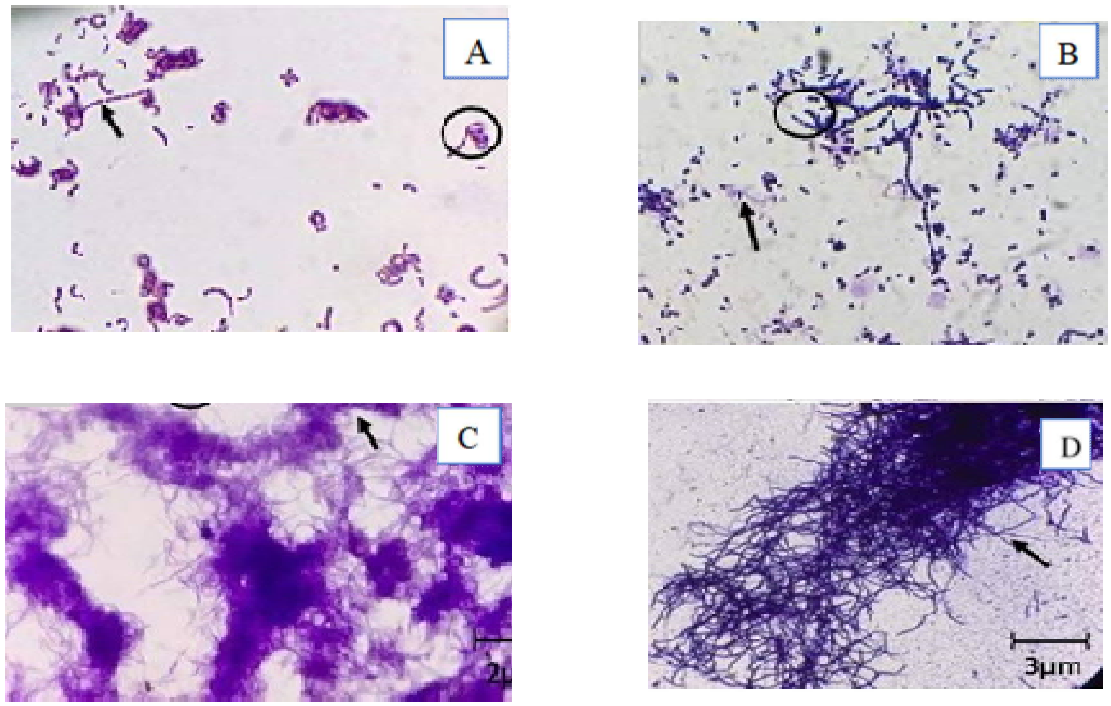


Figure 3. The inhibitory zone of actinomycetes against *E.coli* ATCC25922 *by* disk diffusion method. The code of A) VM 04, (B) VM03,(C) VM 01,and (D) VM 02

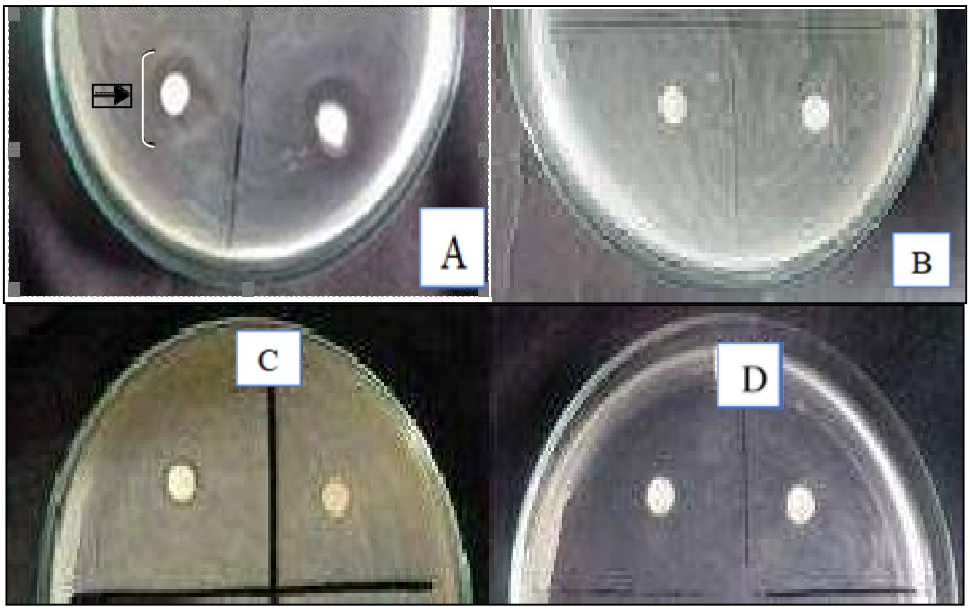
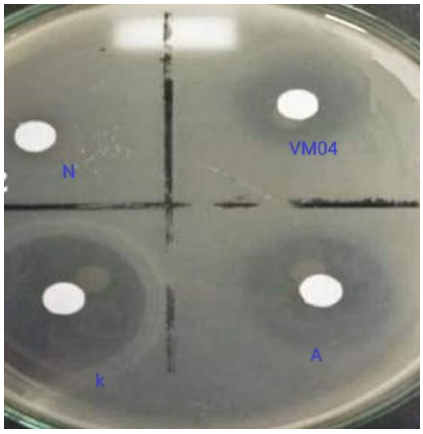


Figure 4: Inhibitory zone VM-04 and positive control, A: Amoxicillin, K: Chloramphenicol against *E. coli* ATCC25922



DISCUSSION

The diversity of actinomycetes is significantly influenced by the physical and chemical properties of soil [[17]]. Actinomycetes are a source of bioactive compounds with antibacterial, antifungal, antiviral, and antiparasitic action, according to empirical evidence [17]. According to [18], several actinomycetes that generate antimicrobial chemicals are aerobic chemoorganotrophic, contain oxidative metabolites, and draw energy from a variety of carbon sources. Actinomycetes may grow at their best at temperatures between 28 and 32 °C. Actinomycetes grow best at temperatures between 28 and 32 °C, according to [19]. According to [20]], actinomycetes may grow in the pH range of 4-5 and thrive in 6.5-8.0. The actinomycetes used in this investigation were found in soil that had a pH of 4. Location I, which had a pH of 4-6 and a salinity of 2%–15%, was able to effectively isolate four actinomycetes. At site II, which had 0% salinity and the same pH, there were no actinomycetes. It shows that actinomycetes' development is not solely influenced by pH. Environmental variables such as temperature, pH, salinity, dissolved oxygen, dissolved nitrate, dissolved nitrite, dissolved phosphate, and dissolved ammonia all had an impact on the density of actinomycetes. Environmental factors can potentially reduce the variety of actinomycetes in a given area. The existence and distribution of actinomycetes' habitat are influenced by the physical and chemical characteristics of the soil [21]. The majority of actinomycetes are found in wet and coastal soil. It is because actinomycetes may grow and reproduce under certain environmental circumstances. In biological applications including antibacterial, anticancer, antifungal, and enzyme makers, marine actinomyces play a significant part [21]. Actinomycetes cannot develop in several environmental circumstances, including excessive salinity, acidic environments, and extremely high temperatures. However, certain actinomycetes have been discovered in coastal soils, which may provide the proper environment and nutrients for their development. Under ideal climatic conditions, actinomycetes generate the best antibiotics [8], [17]. However, the salinities from both locations were not too high in this investigation, although just a few isolates were collected. Site I yielded four isolates (VM 01, VM 02, VM03, and VM04), but site II yielded none of the actinomycetes isolates. The soil at position II had an unpleasant odor and a light brown color that may make actinomycetes undesirable. In a prior investigation, [22] discovered 10 isolates of actinomycetes strains in mangrove sediments in Egypt's Red Sea. In addition, thirty actinomycetes have been isolated from Antarctica [23]. In comparison, [[24]] identified actinomycetes from Nipah worm pelleted feces and the digestive tract. It demonstrates that actinomycetes may be isolated from a variety of habitats as long as the environment fits the nutritional demands of actinomycetes [24]. Actinomycetes isolated from the vellar estuary's mangrove rhizosphere soil, Parangipettai, belonging to the Streptomyces and Saccharopolyspora spp. genera (Table 3) inhibited the development of *E. coli* but not S. aureus or K. pneumoniae. Previous research has shown that actinomycetes can limit the development of *Bacillus, Staphylococcus, E. coli, Klebsiella, and Pseudomonas*[25]. Streptomyces is one of the Actinomycetes genera that generates the most antibiotics.

Streptomyces antibiotics are often streptomycin generated by *S.griceus,* which can inhibit most Gram-negative bacteria. *Streptomyces spp.* generates spectinomycin, which inhibits Mycobacterium TB growth. *S. fradiae* generates broad-spectrum antibiotics such as neomycin. *S. aurefaciens* produces tetracycline, which inhibits Gram-positive and Gram-negative bacteria with a broad range, including Rickettsia's. Gram-negative bacteria are inhibited by erythromycin, which is generated by *S. erythroid*. S. Venezuela chloramphenicol has a limited antibiotic range, whereas Streptomyces generates many more antibiotics. The genus Streptomyces produces around 60 different antibiotics. VM -04 was the isolate that generated

crude antimicrobial compounds (CACs) with the greatest inhibition against *E. coli*. Overall, the actinomycetes isolated from the mangrove rhizosphere soil in Vellar Estuary Parangipettai show antibacterial activity against *E. coli*, according to this study. As a result, *Streptomyces* spp strain VM-04 might be developed as an antibacterial agent.

CONCLUSION

An effort was made to identify several strains of actinomycetes with antibacterial activity from the mangrove rhizosphere soil in the Vellar estuary, Parangipettai. There is currently a need to discover novel antimicrobial-producing strains since existing medications have failed owing to the development of resistance among microbes. The current study is also a little contribution to this requirement. Isolates that demonstrated broad-spectrum effectiveness against the test pathogens might be investigated as possible antibacterial chemical compounds.

REFERENCES

1. K. S. Almaary *et al.*, (2021). "Anti-bacterial effect of marine sea grasses mediated endophytic actinomycetes against K. pneumoniae," *J King Saud Univ Sci*, vol. 33, no. 6, p. 101528, doi: 10.1016/j.jksus.2021.101528
2. E. Ancheeva, G. Daletos, and P. Proksch, (2018). "Lead Compounds from Mangrove-Associated Microorganisms," *Mar Drugs*, vol. 16, no. 9, p. 319, doi: 10.3390/md16090319.
3. K. Palit, S. Rath, S. Chatterjee, and S. Das, (2022). "Microbial diversity and ecological interactions of microorganisms in the mangrove ecosystem: Threats, vulnerability, and adaptations," *Environmental Science and Pollution Research*, vol. 29, no. 22, pp. 32467–32512. doi: 10.1007/s11356-022-19048-7.
4. H. Gao *et al.*, (2021). "Bioactive Metabolites from Acid-Tolerant Fungi in a Thai Mangrove Sediment," *Front Microbiol*, vol. 11, doi: 10.3389/fmicb.2020.609952
5. J. W.-F. Law *et al.*, (2019). "Diversity of Streptomyces spp. from mangrove forest of Sarawak (Malaysia) and screening of their antioxidant and cytotoxic activities," *Sci Rep*, vol. 9, no. 1, p. 15262. doi: 10.1038/s41598-019-51622-x.
6. E. O. Ibnouf, (2021). "Screening of O-7 Isolate Actinomycete Producing Antimicrobials in Different Growth Conditions against Selected Pathogens," *International Journal Of Pharmaceutical And Phytopharmacological Research*, vol. 11, no. 2, pp. 13–23. doi: 10.51847/WaOnDShxEP.
7. Raja and P. Prabakaran, (2011). "Actinomycetes and Drug-An Overview," *American Journal of Drug Discovery and Development*, vol. 1, no. 2, pp. 75–84. doi: 10.3923/ajdd.2011.75.84.
8. Shrestha, D. K. Nath, A. Maharjan, A. Poudel, R. N. Pradhan, and S. Aryal, (2021). "Isolation and Characterization of Potential Antibiotic-Producing Actinomycetes from Water and Soil Sediments of Different Regions of Nepal," *Int J Microbiol*, vol. pp. 1–9, doi: 10.1155/2021/5586165.
9. J. Bérdy, (2012). "Thoughts and facts about antibiotics: Where we are now and where we are heading," *J Antibiot (Tokyo)*, vol. 65, no. 8, pp. 385–395. doi: 10.1038/ja.2012.27.
10. Wahab, S. Shumaila, S. A. Subhan, S. T. Ali, and T. Y. Mujahid, (2015). "Isolation and identification of actinomycetes isolated from Karachi soil and screening of antimicrobial compounds," *Int J Curr Res*, vol. 7, no. 2, pp. 12760–12765.
11. W. Zhang *et al.*, (2021). "Diversity patterns and drivers of soil microbial communities in urban and suburban park soils of Shanghai, China," *PeerJ*, vol. 9, p. e11231, doi: 10.7717/peerj.11231.
12. C. Sweetline, R. Usha, and M. Palaniswamy, "Antibacterial activity of actinomycetes from Pichavaram Mangrove of Tamil Nadu," *Applied Journal of Hygiene*, vol. 1, no. 2, pp. 15–18, 2012
13. J. E. L. Daquioag and G. M. Penuliar, "Isolation of Actinomycetes with Cellulolytic and Antimicrobial Activities from Soils Collected from an Urban Green Space in the Philippines," *Int J Microbiol*, vol. 2021, pp. 1–14, Mar. 2021, doi: 10.1155/2021/6699430.
14. H. Krismawati, L. Sembiring, and S. Wahyuono, "Antibiotic-producing streptomycetes associated with the rhizosphere of several mangrove species," *Plasma: Jurnal Kesehatan*, vol. 1, no. 2, pp. 59–70, 2015.
15. Parte *et al.*, *Bergey's manual of systematic bacteriology: volume 5: the Actinobacteria*. Springer Science & Business Media, 2012.
16. D. H. Bergey, (1994). *Bergey's manual of determinative bacteriology*. Lippincott Williams & Wilkins.
17. V. Raval and N. S. Sahay, (2021). "Isolation of microbes from Valley of Flower (VOF) India and screening of actinomycetes for their antibiotic potential," *Intl J Ind Biotechnol Biomater*, vol. 7, no. 1, pp. 9–21.
18. J.-C. Bertrand, P. Caumette, P. Lebaron, R. Matheron, P. Normand, and T. S. Ngando, (2015). *Environmental microbiology: fundamentals and applications*. Springer.
19. B. R. Tiwari, T. Rouissi, S. K. Brar, and R. Y. Surampalli, (2021). "Critical insights into psychrophilic anaerobic digestion: Novel strategies for improving biogas production," *Waste Management*, vol. 131, pp. 513–526, doi: 10.1016/j.wasman.2021.07.002.
20. M. Prasad, S. Kumar, and U. Kumar, "Anupama. (2015). Screening of endophytic actinomycetes from different indigenous medicinal plants," *Eur J Exp Biol*, vol. 5, no. 4, pp. 7–14.
21. M. S. M. Selim, S. A. Abdelhamid, and S. S. Mohamed, (2021). "Secondary metabolites and biodiversity of actinomycetes," *Journal of Genetic Engineering and Biotechnology*, vol. 19, no. 1, p. 72. doi: 10.1186/s43141-021-00156-9.

22. M. M. Hamed *et al.*, (2021). "Distribution and Characterization of Actinomycetes in Mangrove Habitats (Red Sea, Egypt) with Special Emphasis on Streptomyces mutabilis M3MT483919," *J Pure Appl Microbiol*, vol. 15, no. 1, pp. 246–261, Mar. doi: 10.22207/JPAM.15.1.19.
23. L. J. Silva *et al.*, (2020). "Actinobacteria from Antarctica as a source for anticancer discovery," *Sci Rep*, vol. 10, no. 1, p. 13870, doi: 10.1038/s41598-020-69786-2.
24. H. Yanti, T. R. Setyawati, and R. Kurniatuhadi, "(2020). Composition and Characterization of Actinomycetes Isolated from Nipah Mangrove Sediment, Gastrointestinal and Fecal Pellets of Nipah Worm (*Namalycastis Rhodhocorde*)," *IOP Conf Ser Earth Environ Sci*, vol. 550, no. 1, p. 012003, doi: 10.1088/1755-1315/550/1/012003.
25. Ozer, A. Kalaci, E. Semerci, N. Duran, S. Davul, and A. N. Yanat, (2010). "Infections and aerobic bacterial pathogens in diabetic foot," *Afr J Microbiol Res*, vol. 4, no. 20, pp. 2153–2160.