

Investigation of Halophilic Actinomycetes: Isolation, Identification, and Antibacterial Activity against MDR Pathogens

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Abstract

Halophilic actinomycetes from saline environments represent a promising yet comparatively underexplored source of antibacterial metabolites, particularly against multidrug-resistant (MDR) bacterial pathogens. The present study aimed to isolate and characterize halophilic actinomycetes from saltpan soil collected from Vedaranyam, Nagapattinam district, Tamil Nadu, and evaluate their antibacterial potential against selected MDR pathogens. The collected soil exhibited a slightly alkaline pH (7.40), high electrical conductivity (11.20 dS m⁻¹), and salinity of 2.14 g L⁻¹. A total of 27 presumptive actinomycete isolates were recovered, of which 12 isolates exhibited tolerance to 5–10% NaCl and were selected for further screening. Preliminary antibacterial screening against *Escherichia coli*, *Staphylococcus aureus*, *Corynebacterium striatum*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* identified five active isolates, namely HA2, HA5, HA8, HA10 and HA12. Among these, HA5 showed the highest antibacterial activity at 100 µg mL⁻¹, with inhibition zones of 11 ± 0.76, 13 ± 1.83, 16 ± 0.74, 17 ± 0.58, 19 ± 1.00 and 15 ± 0.76 mm against *E. coli*, *S. aureus*, *C. striatum*, *S. pneumoniae*, *P. aeruginosa* and *E. faecalis*, respectively. Based on its superior antibacterial activity, HA5 was selected for secondary metabolite characterization. GC–MS analysis of the ethyl acetate crude extract revealed 25 tentatively identified compounds, with relatively abundant constituents including 1H-Purin-6-amine, 2-fluorophenyl methyl, 6-octadecenoic acid methyl ester, squalene, N-hexadecanoic acid, oleic acid and 3-tetradecanol. The findings demonstrate that Vedaranyam saltpan soil is a valuable source of salt-tolerant actinomycetes with broad-spectrum antibacterial potential. In particular, HA5 represents a promising candidate for further investigation of its bioactive secondary metabolites and their potential application against MDR bacterial pathogens.

Keywords: Halophilic actinomycetes; saltpan soil; *Streptomyces* sp.; MDR pathogens; antibacterial activity; secondary metabolites; GC–MS; Vedaranyam.

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Introduction

Antimicrobial resistance (AMR) is now a major public health concern as it reduces the efficacy of treatments against bacterial infections. This has created a increased demand for new antimicrobial drugs. Antibiotic resistance, as part of AMR, has led to treatment failures and constrained the use of a number of commonly used antibiotics. The new resistance also lags behind the development of new antibacterial agents. The burden of AMR caused by bacteria has been massive with high levels of global mortality and morbidity. The latest version of the WHO Bacterial Priority Pathogens List cites a mounting need for the development of new antibacterial drugs targeted against bacteria with clinically significant resistance (Murray et al., 2022; Sati et al., 2025).

Actinomycetes are an ecologically and metabolically diverse group of filamentous, mainly Gram-positive, bacteria that produce a wide variety of bioactive, structurally diverse secondary metabolites. In contrast to many other bacteria, which are found in terrestrial and aquatic environments, soils have been the traditional source of Actinomycetes. Many soils, however, contain high amounts of salinity, and there are several salinity-tolerant environments that have not yet been explored for their microbial diversity. Saline environments contain a number of halophilic and halotolerant Actinomycetes isolated from marine sediments, solar salterns, saline soils, and other very saline environments. These microorganisms, which can tolerate high salt concentrations or even require them for growth, possess biosynthetic potential

typical for Actinomycetes, and thus represent promising organisms for the discovery of new bioactive compounds (Hamed et al., 2013; Manivasagan et al., 2014).

Halophilic actinomycetes make up a physiological group that has relatively few studies have been carried out on it, and the way they have adapted to saline environments might give access to special metabolic resources. Over 70 species of halophilic and halotolerant actinomycetes from a number of genera have been identified, showing the taxonomic diversity found in this group. However, only a portion of these micro-organisms have been examined for their capacity to produce secondary metabolites, which indicates a great deal of untapped potential for the discovery of natural products. Because of the combination of their biosynthetic ability and their adaptation to extremely saline conditions, halophilic actinomycetes are especially appealing targets for antimicrobial bioprospecting (Hamed et al., 2013; Jose and Jebakumar, 2013).

The appearance of multidrug-resistant (MDR) pathogens has greatly complicated the treatment of bacterial infections. Since these resistant bacteria can be resistant to several groups of antimicrobial agents, the number of available treatment options is restricted and the chance of treatment failing increases. Examples of clinically significant resistant pathogens are carbapenem-resistant *Acinetobacter baumannii*, carbapenem-resistant and third-generation-*cephalosporin-resistant*

Enterobacterales, *carbapenem-resistant Pseudomonas aeruginosa*, *methicillin-resistant Staphylococcus aureus* and *vancomycin-resistant Enterococcus faecium*. These organisms are included among the critical and high-priority resistant pathogens in the WHO 2024 Bacterial Priority Pathogens List, which shows how important they are for antimicrobial research and development (WHO, 2024; Sati et al., 2025).

The growing prevalence of MDR pathogens has made it necessary to find new antibacterial compounds with different structures and mechanisms of action. Natural products derived from microorganisms, especially actinomycetes, still act as a significant source for the discovery of antibacterial agents and could yield chemically diverse molecules that are able to target resistant bacterial pathogens (Tacconelli et al., 2018; Sati et al., 2025).

The formation of secondary metabolites by actinomycetes is greatly affected by environmental and ecological factors such as the availability of nutrients, the physicochemical conditions and their interactions with other microorganisms. Cues from the environment can cause biosynthetic gene clusters to be expressed and lead to the production of metabolites which would probably be only weakly expressed under normal laboratory conditions. The special physiological features of

halophilic actinomycetes can thus lead to the production of unusual metabolites and increase the chemical diversity that can be accessed via microbial bioprospecting. Halophilic actinomycetes therefore represent a promising strategy for the discovery of new bioactive secondary metabolites, especially antibacterial ones (Hamed et al., 2013; van der Meij et al., 2017).

Molecular profiling has been successful in actinomycetes from saline and hypersaline environments. For example, molecular fingerprinting approaches have been employed to cluster actinomycetes isolated from hypersaline solar salterns and then, 16S rRNA gene sequence-based phylogenetic analysis has been performed to identify them. These methods allow researchers to determine the taxonomic diversity of saline actinomycetes and to isolate phylogenetically distinct strains showing antimicrobial activity. Integrating phenotypic characterization with molecular profiling therefore provides a robust strategy for selecting and identifying promising halophilic actinomycetes for further investigation of their antibacterial potential (Jose and Jebakumar, 2013; Ballav et al., 2015).

The present study was undertaken to isolate and characterize halophilic actinomycetes from saltpan soil of Vedaranyam, Nagapattinam district, Tamil Nadu, and to evaluate their antibacterial potential against multidrug-resistant (MDR) bacterial pathogens. The study also aimed to identify promising halophilic actinomycete isolates through phenotypic and molecular profiling, and to characterize the secondary metabolites produced by the selected potent isolate using gas chromatography-mass spectrometry (GC-MS).

Materials and methods

Collection of Soil Samples

Saltpan soil samples were collected from the Vedaranyam saltpan region, Nagapattinam district, Tamil Nadu, India. Soil samples were collected aseptically from a depth of approximately 10 cm below the soil surface to minimize contamination from surface-associated materials. The collected samples were transferred into sterile containers and transported to the laboratory for further processing and isolation of halophilic actinomycetes. Similar sampling and isolation approaches have been reported for the recovery of actinomycetes from Vedaranyam saltpan environments (Deepa et al., 2012; Vigneshwari et al., 2022).



Fig. 1. Sampling location of saltpan soil at Vedaranyam, Nagapattinam district, Tamil Nadu, India.

Physicochemical Analysis of Soil Samples

The collected saltpan soil samples were subjected to physicochemical analysis to determine their major environmental characteristics. The pH, electrical conductivity (EC), salinity and selected mineral/nutrient constituents, including calcium, sodium, phosphate, potassium and nitrogen, were determined using standard soil analytical procedures. The pH and electrical conductivity were measured using appropriate calibrated instruments after preparation of the soil suspension/extract. The obtained physicochemical characteristics were used to assess the saline nature of the sampling site and to relate the environmental conditions with the occurrence of halophilic actinomycetes.

Enumeration and Isolation of Halophilic Actinomycetes

The collected soil samples were processed using the serial dilution and spread-plate technique. Appropriate soil dilutions were prepared using sterile diluent, and aliquots were inoculated onto Actinomycetes Isolation Agar. The inoculated plates were incubated under suitable conditions until distinct actinomycete-like colonies developed. Actinomycete colonies were recognized based on characteristic morphological features such as dry, powdery, or leathery texture, pigmentation, colony colour, irregular margins and characteristic earthy odour. Morphologically distinct colonies were selected for further purification. The use of selective isolation media and dilution plating is a commonly employed approach for recovering actinomycetes

from saline and hypersaline soils (Jose & Jebakumar, 2013; Vigneshwari et al., 2022).

Screening for Halophilic Nature

The salt tolerance of the purified actinomycete isolates was evaluated by growing the isolates on Starch Casein Agar supplemented with different concentrations of sodium chloride (NaCl). The isolates were inoculated onto media containing increasing NaCl concentrations and examined for their ability to grow under elevated salinity. Isolates exhibiting growth at 5–10% NaCl were considered salt-tolerant/halophilic candidates and selected for further investigation. The use of NaCl-amended media for differentiating halophilic and halotolerant actinomycetes has been previously described (Hamed et al., 2013; Jose & Jebakumar, 2013).

Preliminary Screening for Antagonistic Activity

The Cross-streak method was used to screen the isolated halophilic actinomycetes for bacterial pathogens in order to determine their antibacterial activity. After streaking the actinomycetes on the center of the starch casein agar, they were cultured for seven days. Following the incubation, the multidrug-resistant bacterial pathogens (*E.coli*, *Staphylococcus aureus*, *Corynebacterium striatum*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*) were streaked on the plate sidewalls and reincubated for 24 hours at 37°C. The antimicrobial-producing halophilic actinomycetes were chosen for additional research based on the existence or lack of an inhibitory zone (Velho-Pereira & Kamat, 2012).

Secondary Screening of Cell-Free Supernatants

The promising actinomycete isolates were further subjected to secondary screening to determine whether extracellular antibacterial metabolites were released into the culture supernatant. The selected isolates were cultivated under appropriate fermentation conditions, after which the cultures were separated to obtain cell-free supernatants. The supernatants were tested against the selected MDR bacterial pathogens using the agar well-diffusion method. The agar well diffusion method was used to assess the antibacterial activity of the cell-free supernatant. Nutrient broth was used to inoculate pathogenic bacterial strains, and the culture was allowed to grow exponentially overnight at 37°C. Additionally, Mueller-Hinton agar plates were swabbed with the bacterial cultures, creating a 6 mm well. The wells were filled with varying quantities of cell-free culture supernatant (25, 50, 75, and 100 µL) and incubated for 24 hours at 37 degrees Celsius. Lastly, the antibiotic zone scale was used to evaluate the zone of inhibition (Jose & Jebakumar, 2013; Vigneshwari et al., 2022).

Identification and Microbiological Characterization

Based on the primary and secondary screening of antibacterial activity, the selected strain was subjected to physiological, cultural and biochemical

characterization for preliminary identification. Physiological characteristics were evaluated by Gram staining, motility, colony morphology, spore arrangement, aerial mycelium and spore colour, and melanin pigment production. Cultural characteristics were assessed by observing growth and colony pigmentation on Starch Casein Agar, Actinomycetes Isolation Agar, Oatmeal Agar, Kenknight and Muaier's Medium Agar, Glycerol Asparagine Agar, Yeast Malt Extract Agar and Streptomyces Project Medium (ISP). Biochemical characterization was performed using standard tests for indole, methyl red, Voges-Proskauer, triple sugar iron, urease, nitrate reduction, catalase, oxidase, starch hydrolysis, casein hydrolysis, and gelatin hydrolysis. Carbohydrate utilization was determined using glucose, fructose, sucrose, and maltose.

Preparation of Crude Secondary Metabolite Extract

The isolate exhibiting the most promising antibacterial activity was selected for secondary-metabolite extraction. The culture broth was separated from the microbial biomass, and the cell-free supernatant was subjected to solvent extraction using ethyl acetate, Petroleum ether, hexane, and Chloroform. The organic phase containing the extracellular metabolites was separated and concentrated to obtain the crude extracts. The resulting crude extracts were used for antimicrobial activity. The antibacterial activity of the active fractions obtained from the isolate was evaluated against the selected bacterial pathogens using the agar well diffusion method (Vigneshwari et al., 2022). The positive control is chloramphenicol, and the negative control is DMSO. The zone of inhibition was measured in mm.

GC-MS Characterization of Secondary Metabolites

The chemical constituents present in the ethyl acetate crude extract of the selected halophilic actinomycete isolate were analyzed by gas chromatography-mass spectrometry (GC-MS). The chromatographic peaks obtained from the extract were recorded based on their retention time and peak area. The mass spectra corresponding to individual peaks were compared with entries in the NIST mass spectral library for tentative identification of the detected compounds. The identified compounds were tabulated according to their compound name, retention time and relative peak area. Compounds showing comparatively higher peak areas were considered major constituents of the crude extract and were selected for further consideration. GC-MS-based chemical profiling has previously been employed for characterization of bioactive metabolites produced by halophilic actinomycetes isolated from the Vedaranyam saltpan environment (Vigneshwari et al., 2022).

Results

Physicochemical Characteristics of Saltpan Soil

The physicochemical characteristics of the Vedaranyam saltpan soil sample are presented in Fig. 2. The soil exhibited a slightly alkaline pH of 7.40 and a high electrical conductivity (EC) of 11.20 dS m⁻¹, indicating a saline soil environment. The measured salinity was 2.14 g L⁻¹. Among the analyzed mineral and nutrient constituents, calcium was recorded at 6.23 mg kg⁻¹, whereas sodium represented the predominant measured constituent at 2.89%. Phosphate and potassium contents were 2.80% and 0.72%, respectively. The nitrogen content was comparatively lower at 0.30%. Overall, the physicochemical profile confirmed the saline characteristics of the collected Vedaranyam saltpan soil, providing a suitable environment for the occurrence of salt-tolerant microorganisms.

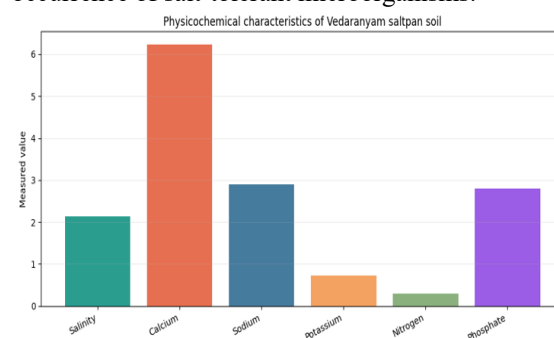


Fig. 2. Physicochemical characteristics of the collected Vedaranyam saltpan soil sample.

Enumeration of Halophilic actinomycetes from saltpan soil

Pretreated saltpan soil samples were serially diluted and plated on actinomycetes isolation agar. After incubation, colonies exhibiting typical actinomycete characteristics, particularly a dry and powdery appearance, were observed and enumerated. Based on colony morphology, including powdery texture, characteristic soil-like odour, pigmentation, colony colour, and shape, a total of 27 presumptive halophilic actinomycete isolates were selected.

The selected colonies were purified by repeated quadrant streaking to obtain morphologically uniform cultures. The purified isolates were subsequently maintained on starch casein agar slants for further characterization and experimental studies.

Isolation and Screening of Halophilic Actinomycetes

A total of 27 actinomycete isolates obtained from the Vedaranyam saltpan soil were screened for their tolerance to increasing concentrations of NaCl using Starch Casein Agar. Among the 27 isolates, 12 isolates exhibited visible growth at NaCl concentrations ranging from 5% to 10%, indicating their ability to tolerate elevated salinity. These 12 salt-tolerant isolates were selected for further evaluation of their antibacterial activity against the selected MDR bacterial pathogens.

Preliminary Screening of Antagonistic Activity

A total of 12 salt-tolerant actinomycete isolates were screened for their antagonistic activity against the selected MDR bacterial pathogens. Among the tested isolates, five isolates, namely HA2, HA5, HA8, HA10, and HA12, exhibited antibacterial activity against all the tested pathogens. The intensity of inhibition varied among the isolates and pathogens, indicating differences in their antibacterial potential. Based on their broad-spectrum antagonistic activity, these five isolates were considered promising candidates for further investigation.

Table 1. Preliminary antagonistic activity of selected halophilic actinomycetes against MDR bacterial pathogens

S. No.	Iso- lat	<i>E. coli</i>	<i>S. aureus</i>	<i>C. striatum</i>	<i>S. pneumoniae</i>	<i>P. aeruginosa</i>	<i>E. faecalis</i>
1	H A1	–	–	–	–	–	–
2	H A2	+	+	+	+	+	+
3	H A3	–	–	–	–	–	–
4	H A4	–	–	–	–	–	–
5	H A5	+	++	++	++	++	++
6	H A6	–	–	–	–	–	–
7	H A7	–	–	–	–	–	–
8	H A8	+	+	+	+	+	+
9	H A9	–	–	–	–	–	–
10	H A10	+	+	+	+	+	+
11	H A11	–	–	–	–	–	–

1	H	+	+	+	+	+	+
2	A1						
2							

– : No inhibition, + : moderate inhibition, ++ : high inhibition

Secondary Screening of Cell-Free Supernatants

The antibacterial activity of the five selected halophilic actinomycete isolates was evaluated against six MDR bacterial pathogens at a concentration of 100 µg/mL, and the results are presented in Table 2. All five isolates exhibited antibacterial activity against the tested pathogens, with inhibition zones ranging from 8 ± 0.89 to 19 ± 1.00 mm. The extent of inhibition varied considerably among the isolates and test pathogens. Among the tested isolates, HA5 exhibited the highest overall antibacterial activity, producing inhibition zones of 11 ± 0.76 mm against *E. coli*, 13 ± 1.83 mm against *S. aureus*, 16 ± 0.74 mm against *C. striatum*, 17 ± 0.58 mm against *S. pneumoniae*, 19 ± 1.00 mm against *P. aeruginosa*, and 15 ± 0.76 mm against *E. faecalis*. The maximum inhibition produced by HA5 was observed against *P. aeruginosa* (19 ± 1.00 mm), followed by *S. pneumoniae* (17 ± 0.58 mm) and *C. striatum* (16 ± 0.74 mm).

HA8 also demonstrated substantial antibacterial activity, with inhibition zones ranging from 10 ± 1.00 to 17 ± 0.58 mm, showing its highest activity against *P. aeruginosa*. HA2 produced inhibition zones ranging from 9 ± 1.00 to 14 ± 0.86 mm, whereas HA12 showed inhibition zones ranging from 10 ± 0.78 to 14 ± 1.65 mm. HA10 exhibited inhibition zones ranging from 8 ± 0.89 to 15 ± 0.89 mm, with the lowest activity observed against *E. coli*.

Based on its consistently higher inhibition zones against all six MDR pathogens, HA5 was identified as the most promising isolate and was selected for subsequent secondary metabolite extraction and GC–MS characterization.

Table. 2. Secondary screening of cell-free supernatant of strains against MDR bacterial pathogens

Value represents mean $\pm$ SD;n=3,

Biochemical Identification of Isolate HA5

The biochemical characteristics of the potent halophilic actinomycete isolate HA5 were evaluated to support its phenotypic identification. The isolate exhibited a typical Gram-positive (Figure 3) and was positive for catalase, oxidase, citrate utilization, urease, nitrate reduction, starch hydrolysis, casein hydrolysis, and gelatin hydrolysis. The isolate was negative for indole, methyl red, Voges-Proskauer, and hydrogen sulfide production. HA5 also demonstrated growth at elevated NaCl concentrations, confirming its salt-tolerant nature. The overall biochemical profile was consistent with characteristics commonly observed in members of the genus *Streptomyces* (Table 3,4).

Table. 3. Physiological and cultural characteristics of HA5 strain

S . No	Iso lat es	Concentration (100 µg/mL)/ Zone of Inhibition (mm)					
		<i>E. coli</i>	<i>S. aureus</i>	<i>C. striatum</i>	<i>S. pneumoniae</i>	<i>P. aeruginosa</i>	<i>E. faecalis</i>
	H A2	9 $\pm$ 1.0	14 $\pm$ 0.86	13 $\pm$ 0.58	11 $\pm$ 1.00	9 $\pm$ 1.00	14 $\pm$ 0.86
	H A5	11 $\pm$ 0.76	13 $\pm$ 1.83	16 $\pm$ 0.74	17 $\pm$ 0.58	19 $\pm$ 1.00	15 $\pm$ 0.76
	H A8	10 $\pm$ 1.00	12 $\pm$ 1.12	14 $\pm$ 0.58	15 $\pm$ 1.15	17 $\pm$ 0.58	11 $\pm$ 1.00
	H A10	8 $\pm$ 0.89	11 $\pm$ 0.63	15 $\pm$ 0.89	14 $\pm$ 0.65	12 $\pm$ 0.21	10 $\pm$ 0.89
	H A12	10 $\pm$ 0.78	12 $\pm$ 0.32	14 $\pm$ 0.65	14 $\pm$ 0.42	13 $\pm$ 1.68	11 $\pm$ 0.78

S.No	Test/Culture media	HA5	9) <i>Streptomyces</i> Project medium (ISP)	Whitish grey
1)	Gram staining	+		
2)	Motility	-		
3)	Colony form	Circular		
4)	Spores arrangement	Short chain of spores		
5)	Aerial mycelium and colour of the spore	Whitish grey		
6)	Starch casein agar	Pure white		
7)	Actinomycetes Isolation agar	Grey white		
8)	Yeast malt extract agar	Greyish brown		



Fig 4: Gram's staining of HA5

Table. 4. Biochemical Characterization of Halophilic strains

Antibacterial activity of crude extracts

The antibacterial activity of HA5 and its solvent fractions at 100 µg/mL is shown in Table 5. The fractions demonstrated variable antibacterial activity against the tested pathogens, with the highest activity being exhibited by the ethyl acetate fraction. The maximum zone of inhibition was observed against *S. pneumoniae* (16 ± 1.00 mm) and *P. aeruginosa* (16 ± 1.53 mm), followed by *C. striatum* (15 ± 1.21 mm) and *S. aureus* (15 ± 1.00 mm). The chloroform fraction also exhibited considerable activity. Petroleum ether and hexane fractions exhibited comparatively lower activity. *E. faecalis* was found to be least susceptible, and activity was observed only in hexane, chloroform and ethyl acetate fractions. Among the fractions tested, the ethyl acetate fraction exhibited the most consistent antibacterial activity against the pathogens tested.

Table 5: Antibacterial activity of crude extracts from HA5 strain

S.No	Biochemical Test	HA5	Tentative genera
1.	Indole	Positive	<i>Streptomyces sp</i>
2	Methyl- Red	positive	<i>Streptomyces sp</i>
3	Voges-Proskauer	positive	<i>Streptomyces sp</i>
4	Triple Sugar Iron	positive	<i>Streptomyces sp</i>
5	Urease	Negative	<i>Streptomyces sp</i>
6	Nitrate	positive	<i>Streptomyces sp</i>
7	Catalase	positive	<i>Streptomyces sp</i>
8	Oxidase	positive	<i>Streptomyces sp</i>
9	Starch hydrolysis	positive	<i>Streptomyces sp</i>
10	Casein hydrolysis	positive	<i>Streptomyces sp</i>
11	Gelatin hydrolysis	Negative	<i>Streptomyces sp</i>
12	Glucose	positive	<i>Streptomyces sp</i>
13	Fructose	Negative	<i>Streptomyces sp</i>
14	Sucrose	positive	<i>Streptomyces sp</i>
15	Maltose	positive	<i>Streptomyces sp</i>

Pathogens	Concentrations (100 µg/mL)/Zone of inhibition (mm)			
	Petroleum Ether	Hexane	Chloroform	Ethyl acetate
<i>E. coli</i>	8±0.58	11±1.00	12±0.48	14±1.53
<i>S. aureus</i>	9±1.00	-	10±0.58	15±1.00
<i>C. striatum</i>	9±1.00	-	14±0.65	15±1.21
<i>S. pneumoniae</i>	-	8±1.00	13±1.00	16±1.00
<i>P. aeruginosa</i>	10±1.00	9±0.58	13±0.58	16±1.53

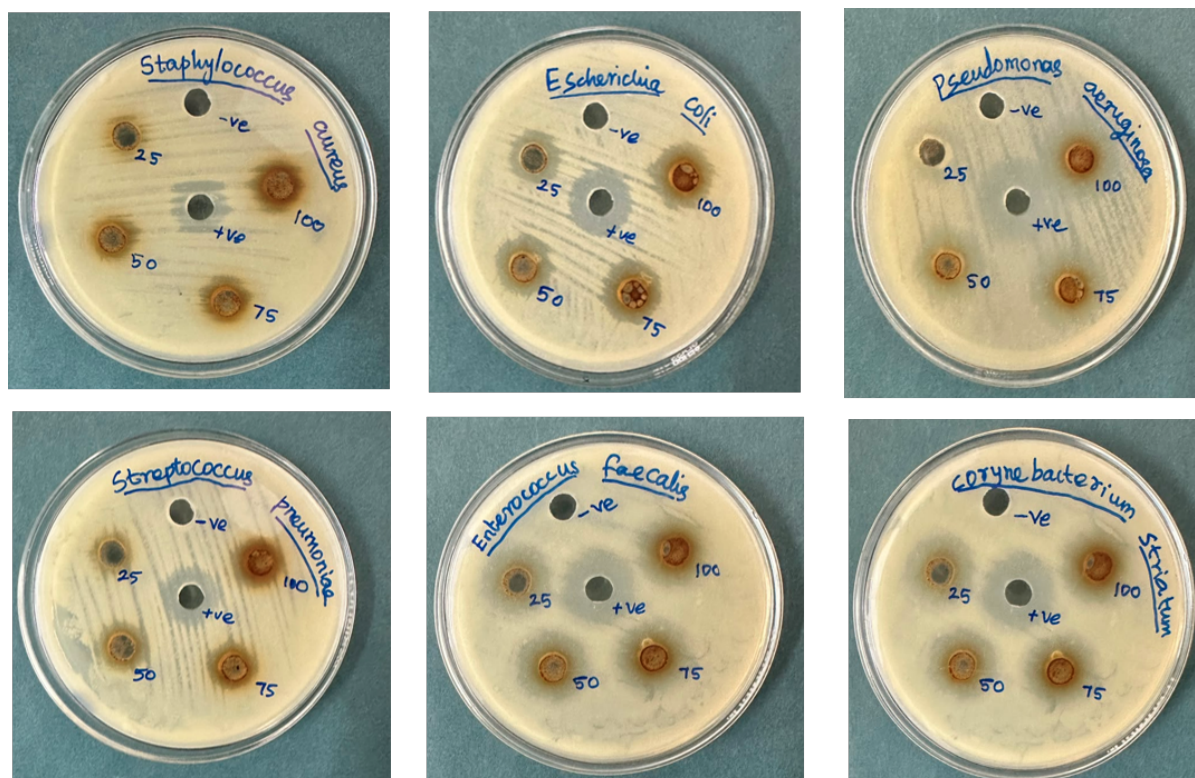
<i>E. faecalis</i>	-	-	11±1.00	15±0.12
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Value represents mean ±SD;n=3,- No zone
Antibacterial activity of the crude fraction isolate extract

The extract of crude fraction isolate from HA5 showed increased antibacterial activity with concentration against all tested pathogens (Table 6, Figure 5). The maximum zone of inhibition was observed against *S. aureus* at 100 µL/mL (24 ± 1.48 mm) followed by *P. aeruginosa* (22 ± 0.58 mm) and *E. coli* (21 ± 1.00 mm) and comparatively lower activity against *C. striatum* (14 ± 1.00 mm). Positive control showed inhibition zones of 24–28 mm and negative control showed no inhibition.

Table 6: Antibacterial activity of the crude fraction isolate extract from HA 5

Pathogens	Concentrations (µL/mL)/Zone of inhibition (mm)
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	25	50	75	100	Pos itiv e	Neg ativ e
<i>E. coli</i>	10± 0.5 8	13± 1.0 0	15± 0.5 8	21± 1.0 0	26± 0.58	-
<i>S. aureus</i>	13± 0.6 4	16± 0.5 8	20± 1.0 0	24± 1.4 8	28± 1.00	-
<i>C. striatum</i>	7±0 .58	10± 1.0 0	12± 0.4 3	14± 1.0 0	26± 0.58	-
<i>S. pneumoniae</i>	12± 1.0 0	14± 0.6 5	16± 1.0 0	19± 1.5 3	27± 1.53	-

<i>P. aeruginosa</i>	13± 1.0 0	17± 1.5 3	19± 1.5 3	22± 0.5 8	24± 1.53	-
<i>E. faecalis</i>	11± 1.4 7	14± 0.5 8	16± 1.0 0	19± 1.3 1	25± 1.00	-

Value represents mean ±SD;n=3,- No zone

Fig. 5. Antibacterial activity of HA5 ethyl acetate extract against MDR bacterial pathogens

GC-MS Characterization of H5

The chemical compositions of isolates' secondary metabolites were identified, and the separated chromatograph was compared using the NIST library. The H5 ethyl acetate GC-MS chromatogram showed the presence of 25 compounds with peak area and retention time. All the compounds were summarized with their peak area and retention time. The compounds with more area of the peak were further focused on in isolation studies. The area of a peak is directly proportional to the amount of the compound that is found in a given mixture. The major compounds are N-Hexadecanoic acid, 1H-Purin-6-amine, 2-fluorophenyl, 6-Octadecenoic acid, methyl ester, Squalene, Oleic Acid and 3-Tetradecanol, majorly detect in the GC-MS spectrum (Fig.6 and Table 7).

Table 7. Chemical composition of H5 Ethyl acetate crude extract

S.No	Compound Name	Area %	Retention Time
1.	N-Hexadecanoic acid	6.59	13.937
2.	6-Octadecenoic acid, methyl ester	7.83	15.293
3.	1H-Purin-6-amine, 2-fluorophenyl methyl	15.26	19.121
4.	Squalene	6.86	19.703
5.	4-Hexen-1-ol, (4E)-, tert-butyl dimethylsilyl	7.12	15.127
6.	Oleic Acid	4.04	15.791
7.	3-Tetradecanol	5.31	15.000

Fig.6: GC-MS Spectrum of H5 crude extract

Discussion

The present study demonstrated that the Vedaranyam saltpan environment harbours actinomycetes with appreciable salt tolerance and antibacterial potential. From the collected saltpan soil, 27 presumptive actinomycete isolates were obtained, of which 12 isolates tolerated NaCl concentrations ranging from 5% to 10%. The recovery of salt-tolerant actinomycetes from this habitat indicates that saline environments can serve as selective niches for microorganisms possessing physiological adaptations to elevated osmotic stress. Saline soils are also increasingly being explored for *Streptomyces* strains with distinctive biosynthetic potential, and genome-mining studies of saline-soil *Streptomyces* have indicated the presence of diverse biosynthetic pathways associated with potentially bioactive metabolites (Khoshakhlagh et al., 2023).

The antibacterial screening revealed considerable differences among the isolates. Five isolates, namely HA2, HA5, HA8, HA10 and HA12, exhibited activity against all six MDR bacterial pathogens tested. Such broad-spectrum activity is important because actinomycetes remain a rich source of antibacterial metabolites with activity against

clinically relevant resistant bacteria. Recent investigations have similarly reported that *Streptomyces* isolates obtained from underexplored environments can exhibit broad-spectrum activity against drug-resistant pathogens. For example, a marine *Streptomyces* sp. isolated from Tamil Nadu showed antibacterial and antibiofilm activity against antimicrobial-resistant clinical pathogens, demonstrating the value of environmental actinomycetes for MDR-pathogen screening (Dhandapani et al., 2022).

Among the active isolates, HA5 showed the highest antibacterial activity, with inhibition zones of 11 ± 0.76 mm against *E. coli*, 13 ± 1.83 mm against *S. aureus*, 16 ± 0.74 mm against *C. striatum*, 17 ± 0.58 mm against *S. pneumoniae*, 19 ± 1.00 mm against *P. aeruginosa* and 15 ± 0.76 mm against *E. faecalis*. The strongest inhibition was therefore observed against *P. aeruginosa*, followed by *S. pneumoniae* and *C. striatum*. The variation in susceptibility among the pathogens may reflect differences in cell-envelope characteristics, permeability and intrinsic resistance mechanisms. Comparable broad-spectrum antibacterial activity has been reported for environmental *Streptomyces* isolates, including strains whose metabolites were active against resistant Gram-positive and Gram-negative bacteria (Thapa et al., 2024; Abdelaziz et al., 2023).

The comparatively strong activity of HA5 against *P. aeruginosa* is particularly noteworthy because this organism is characterized by multiple intrinsic and acquired mechanisms that can reduce susceptibility to antimicrobial compounds. The ability of an environmental actinomycete isolate to inhibit such a pathogen supports further investigation of its extracellular metabolites. However, inhibition-zone measurements alone cannot establish the potency or mechanism of an antibacterial compound. Determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), time-kill kinetics and, where appropriate, antibiofilm activity would provide stronger evidence for the therapeutic potential of the HA5 metabolites. Recent work with marine *Streptomyces* metabolites has demonstrated that combining agar-diffusion assays with MIC, MBC and antibiofilm investigations provides a more comprehensive assessment of activity against resistant pathogens (Dhandapani et al., 2022).

The selection of HA5 for secondary metabolite investigation was supported by its consistently higher inhibition against all six test pathogens. This observation is consistent with the broader recognition that *Streptomyces* species are particularly productive sources of antibacterial secondary metabolites. Recent chemical and metabolic studies have demonstrated considerable

diversity in the antibacterial metabolites produced by different *Streptomyces* strains, emphasizing that metabolite profiles can vary substantially between isolates even when they belong to the same genus (Thapa et al., 2024).

GC–MS analysis of the HA5 ethyl acetate crude extract revealed 25 tentatively identified compounds, demonstrating considerable chemical diversity in the extract. Among the relatively abundant compounds detected were 1H-Purin-6-amine, 2-fluorophenyl methyl, 6-octadecenoic acid methyl ester, 4-hexen-1-ol derivative, squalene, N-hexadecanoic acid, oleic acid and 3-tetradecanol. Similar chemical profiling approaches have been applied to bioactive *Streptomyces* extracts, where GC–MS has revealed mixtures containing fatty acid derivatives, esters, alcohols, hydrocarbons and steroid-related compounds. For example, an ethyl acetate extract of marine *Streptomyces levis* contained numerous chemically diverse constituents detected by GC–MS and exhibited antimicrobial activity against several bacterial and fungal pathogens (Chakraborty et al., 2022).

The detection of several fatty-acid-related compounds in the HA5 extract is noteworthy because lipid-derived metabolites and other small molecules can contribute to the biological properties of crude microbial extracts. Nevertheless, the GC–MS data obtained in the present study should be interpreted cautiously. Library matching provides tentative chemical identification, and the presence of a compound in the chromatogram does not demonstrate that it is responsible for the antibacterial activity. Similar studies have combined GC–MS profiling with antibacterial assays to identify candidate metabolites, but confirmation of individual compounds requires purification followed by analytical techniques such as NMR and comparison with authentic standards (Chakraborty et al., 2022; Dhandapani et al., 2022).

The findings also support the importance of investigating actinomycetes from geographically and environmentally distinct habitats. A recent study of terrestrial *Streptomyces* sp. MNP32 demonstrated broad-spectrum activity against human pathogens, including WHO-listed priority pathogens, while chemical analysis of its bioactive fraction identified major metabolites by GC–MS. Such findings reinforce the concept that environmental *Streptomyces* isolates can provide promising starting points for the discovery of antibacterial metabolites (Konwar et al., 2023).

Interestingly, the present findings are also consistent with a study specifically conducted in the Kodiyakadu saltpan of Vedaranyam, where a

halophilic *Streptomyces* isolate showed antibacterial activity against several pathogenic bacteria and its crude extract was subjected to GC–MS analysis. This provides regional support for the observation that the Vedaranyam saltpan ecosystem can harbour bioactive halophilic actinomycetes. However, the differences in active isolates, inhibition profiles and detected metabolites between studies also emphasize the strain-specific nature of secondary-metabolite production (Vigneshwari et al., 2022).

Conclusion

The present study demonstrated the occurrence of salt-tolerant actinomycetes in Vedaranyam saltpan soil and highlighted their potential as a source of antibacterial metabolites. Among the 27 presumptive actinomycete isolates obtained, 12 demonstrated tolerance to 5–10% NaCl, confirming their adaptation to elevated saline conditions. Five isolates, HA2, HA5, HA8, HA10 and HA12, exhibited broad-spectrum antibacterial activity against the six tested MDR pathogens. HA5 was identified as the most promising isolate, showing the highest inhibition against all the tested pathogens, with the maximum activity observed against *P. aeruginosa* (19 ± 1.00 mm). The GC–MS profile of the HA5 ethyl acetate extract revealed a chemically diverse mixture comprising 25 tentatively identified compounds, indicating substantial secondary-metabolite-producing potential. Overall, the findings suggest that halophilic actinomycetes associated with Vedaranyam saltpans constitute a promising microbial resource for the discovery of antibacterial metabolites targeting MDR pathogens. Further investigations involving MIC/MBC determination, antibiofilm assays, activity-guided fractionation, purification and structural elucidation of the active metabolites are warranted to establish the therapeutic and biotechnological potential of HA5.

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