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Antibacterial and brine shrimp lethality effect of marine actinobacterium *Streptomyces* sp. CAS72 against human pathogenic bacteria

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PEER REVIEW

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Comments

This is a good study in which authors focused on specific area. Marine actinobacteria was isolated from estuary. From this 46 marine actinobacterial strains 23 isolates was isolated and screened. Then secondary metabolites were isolated, characterized, purified and checked its different antimicrobial activites compared with standard antibiotic nalidixic acid.
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ABSTRACT

Objective: To investigate the *in vitro* antibacterial activity against human pathogenic bacteria and brine shrimp lethality bioassay of the marine actinobacterium.
Methods: Forty six marine actinobacterial strains were isolated from sediment samples of Uppanar estuary, Cuddalore, India. Preliminary screening was done by cross-streak method and the potential strain was identified by morphological, chemotaxonomical and molecular methods. Fermentation was done and the metabolite was obtained by liquid-liquid extraction using ethyl acetate and purified by silica gel (100-200 mesh) column chromatography. The purified metabolite was tested for antibacterial activity, minimal inhibitory concentration and brine shrimp lethality bioassay.
Results: Among the forty six strains, CAS72 was found effective against human pathogenic bacteria. The strain CAS72 was identified as *Streptomyces sp*. The purified metabolite exhibited a significant *in vitro* antibacterial activity. The MIC value was also determined against human pathogenic bacteria and a strong cytotoxic activity in brine shrimp lethality assay was observed and the LC_{50} value was 23.5 μg/mL.
Conclusions: The present investigation reveals that the marine actinobacteria are well obtainable in Uppanar estuary environment and it can provide a definite source for novel bioactive metabolites.

KEYWORDS
Actinobacteria, Brine shrimp lethality, Uppanar estuary

1. Introduction

Microbial bioactive molecules are the source for the development of new medicines. Bio-resources of the ocean has exploited over many decades for microbial bioactive molecules to safeguard our lives. The bioactive molecules have wide applications in agriculture, veterinary and pharmaceutical industry. As for as microbes concern, a group of actinobacteria comes within the order of Actinomycetales, have the potential to produce many different biologically active secondary metabolites such as antibiotics, herbicides, pesticides, anti-parasitic

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compounds and enzymes like cellulose, xylanase[1]. Among these compounds, antibiotics are commercial important and therapeutically valued products[2]. Hence, the actinogenic molecules are the threshold for novel drug discovery.

Among the filamentous actinobacteria group, Streptomycetes have been realized as new opportunity for novel drug discovery[3]. These organisms are prominent in producing new secondary metabolites than other common actinobacteria[4]. *Streptomyces* can produce biologically active molecules of pharmaceutical use, as well as the development of other valuable products like enzymes, nutraceuticals and cosmetics[5]. Of these, 75% of the molecules derived from the genus of *Streptomyces* and more than 5 000 known bioactive compounds documented in this genus[6]. In the present study, marine actinobacteria were isolated from the sediment of Uppanar estuary, Cuddalore, India, and tested for its brine shrimp lethality and antibacterial potential against human pathogenic bacteria. In addition, an attempt made to identify the strain up to species level with morphological, physiological and chemo-taxonomical analysis.

2. Materials and methods

2.1. Chemicals and reagents

All the solvents used in the experiment and silica gel for thin layer chromatography (TLC) were obtained from Sigma-Aldrich, USA. All bacteriological media components were purchased from Hi-Media (Mumbai, India). All other chemicals and reagents were extra pure grade.

2.2. Pathogenic strains

The human pathogenic bacteria *viz.*, *Escherichia coli*-MTCC 1610, *Klebsiella pneumoniae*-MTCC 4030, *Listeria monocytogenes*-MTCC 839, *Salmonella enterica*-MTCC 9844, *Shigella flexneri*-MTCC 1457, *Staphylococcus aureus*-MTCC 3160, *Staphylococcus haemolyticus*-MTCC 3383, *Streptococcus pneumoniae*-MTCC 1935, *Vibrio cholerae*-MTCC 3904 and *Vibrio vulnificus*-MTCC 1145 were procured from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India during March 2008 for evaluating the antagonistic potential of the marine actinobacteria. The lyophilized cells were taken from the vials, inoculated in nutrient broth, and incubated at 37 °C for 24 h. After incubation, the human pathogenic bacteria were sub cultured in nutrient agar slants and stored under refrigeration till the next use.

2.3. Sample collection and enrichment

Sediment samples were collected from five different stations of Uppanar estuary, Cuddalore, India during 2008 in summer. The collected sediment samples were transferred in sterile polyurethane bags tightly covered and stored in the refrigerator until use. One gram of each sediment sample was added in 10 mL of sterile distilled water and stirred for 15 min[7]. The suspension had been allowed to stand for 30 min and the supernatant was separated. Prior to isolation, the samples were enriched by a modified method proposed by Shirling[8]. The supernatant was transferred to conical flasks containing 100 mL of sterile starch casein broth. Samples incubated at 28±2 °C for 7 d in static condition with intermittent shaking.

2.4. Isolation, media and culture conditions

Isolation, 0.1 mL of the enriched media was spread plated onto a starch casein agar medium (g/L): starch 10, casein 0.3, KNO_3 2, $NaCl_2$, K_2HPO_4 2, $MgSO_4.7H_2O$ 0.05, and $CaCO_3$ 0.02, $FeSO_4.7H_2O$ 0.01 and agar 18 supplemented with Nystatin 10 μg/mL and Nalidixic acid 25 μg/mL[9,10]. The plates were incubated at 28±2 °C for seven to ten d and colonies appeared on the plate were purified and maintained in SCA slants for further studies. The isolated marine actinobacterial strains were segregated based on their morphological differences, labeled with numbers for individualization and used for preliminary antibacterial screening.

2.5. Preliminary screening for antibacterial activity

Antibacterial activity of the isolated marine actinobacterial strains were tested against the above mentioned human pathogenic bacteria by a modified cross streak method[11]. Single streak of actinobacteria was made on the surface of the Modified Nutrient agar (50% NA+50% SCA) as a straight line in the left side corner of the petriplate and incubated at room temperature (28±2) °C. After observing a good ribbon-like growth of the actinobacteria on the petriplates, the pathogenic bacteria were streaked at right angles to the original streak of the actinobacterial isolates and incubated at (28±2) °C. The zone of inhibition was measured after 24 h and 48 h. A control plate was also maintained without inoculating the actinobacteria to assess the normal growth of the pathogenic bacteria. The marine actinobacterial strain showing highest inhibition was taken for further identification and fermentation studies.

2.6. Identification of potential marine actinobacteria

2.6.1. Morphological and physiological characteristics

The potential strain CAS72 was taken for further identification. Cultural characteristics of the strain CAS72 was studied by growing the strain on various International *Streptomyces* Project media[8]. The morphology of the spore bearing hyphae with entire spore chain was examined under light microscope by using inclined cover slip technique[12]. Spore chain morphology and ornamentation of the spore surface was examined by using scanning electron microscope[13]. The spore mass colour was visually examined and estimated. Analysis of carbon source utilization was done by following the protocol of Pridham and Gottlieb[14].

2.6.2. Chemo-taxonomical analysis

Whole cell hydrolysate was used to analyze the cell wall amino acids and sugars. The diaminopimelic acid isomers and whole cell sugars were analysis and was done by the method of Kämpfer[15]. Based on these analysis the strain CAS72 was tentatively grouped in *Streptomyces* genus. To precise the strain in genus and species level, molecular identification was performed.

2.6.3. Molecular identification of strain CAS72

2.6.3.1. DNA extraction

Genomic DNA extraction was done by modifying the method of Everest *et al*[16]. Actinobacteria strain was grown in 20 mL of TSB media for 7 d. Cells were harvested by centrifugation (8000 r/min for 2 min), washed with 500 μL of 10mmol/L Tris-HCl/1 mmol/L EDTA buffer (pH 7.7) and resuspended in 500 μL TE buffer (pH 7.7). The sample was kept in boiling water bath for 10 min, cooled for 5 min and centrifuged (8000 r/min for 3 min). The supernatant (300 μL) was transferred to a clean tube and stored at 4 °C.

2.6.3.2. PCR amplification

The isolated DNA was purified and amplified according to Karuppiah *et al*[17]. A 50 μL amplification reaction contained 1 μL template DNA (50-200 ng), 5 μL 10×PCR buffer, 1 μL each PCR primer (20 mmol/L) (27F, 1492R), 1 μL dNTP mix (10 mmol/L), 6 μL $MgCl_2$ (25 mmol/L), 2.5 U *Taq* DNA polymerase, 5 μL DMSO (5%) and 29 μL sterile MilliQ water. The reaction conditions were initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 30 seconds and extension at 72 °C for 90 seconds. A final extension was performed at 72 °C for 10 min. Reaction products were electrophoresed on a 1% agarose gel and the bands were visualized with ethidium bromide under UV light.

2.6.3.3. Sequencing and analysis of 16S rRNA gene

The amplified PCR product was purified. Both strands of 16S rRNA gene were sequenced using a Terminator Cycle Sequencing kit-ABI Prism 310 Genetic Analyzer (Applied Biosystems, USA). To assess the similarity of the sequence BLAST (http: //www. ncbi.nlm.nih.gov/blst) program was used and the sequence was submitted in GeneBank.

2.7. Fermentation and extraction of bioactive metabolites

The potential strain CAS72 was grown at (28±2) °C in starch-casein agar for one week. Spores were collected from the slant culture with 10 mL of the same medium broth. 1 mL of the spore suspension was transferred to 100 mL of SGG production medium (Glucose 1.0 g, glycerol 1.0 g, corn steep powder 0.25 g, peptone 0.5 g, soluble starch 1.0 g, yeast extract 0.2 g, $CaCO_3$ 0.3 g, NaCl 0.1 g and pH 7.3) advised by Goodfellow and Fiedler for novel drug synthesis[4]. The media was incubated at (28±2) °C for 6 d on a rotary shaker (120 r/min). After incubation, the culture broth was centrifuged at 10 000×g for 30 min at 4 °C by maintaining all the physicochemical factors at optimum levels for the culture. Growth and production were estimated at the temperature, 30 °C, pH, 7.5 and sodium chloride concentration, 2.0%. Extracellular metabolites extracted from the supernatant by liquid-liquid extraction, the filtrate was transferred aseptically into the conical flask. An equal volume of ethyl acetate was added separately to the cell-free culture filtrate and shaken for 2 h[18]. Metabolite extraction was carried out using the ethyl acetate solvent on the basis of best solubility and maximum antibacterial activity. The metabolite in the solvent was evaporated in a rotary evaporator, lyophilized and stored for further use.

2.8. Purification

The crude metabolite (5 mg) was chromatographed on silica gel (Merck, particle size 0.100-0.200 mm) column (22 ×5 cm) and eluted with chloroform-ethyl acetate (70:30) to give 10 major fractions (Fr SM I-X). The dried residues of all the 10 major fractions were dissolved, each in a specified volume of ethyl acetate, to give 1 mg/mL concentration and tested for their antibacterial activities against the test organisms by well-diffusion method. Among the 10 fractions, SM V fraction exhibited good activity. The active fraction SM V was purified by chromatography on Sephadex LH20 (Ethyl acetate) to obtain fractions SM Va to SM Ve. The fraction SM-Vd was found to possess good antibacterial activity. This active fraction SM-Vd was further purified by chromatography on Sephadex LH20 (Ethyl acetate) which resulted in one distinct compound. The fractions

were scanned in a spectrophotometer (UV-1800-Shimadzu scientific instruments, USA) to determine their absorption spectra of UV-visible light at wavelengths ranging from 200 to 900 nm. All the extracts and fractions were subjected to Thin Layer Chromatography (TLC) on silica gel (60 F 254 MERCK; 25 TLC aluminium sheets 20×20 cm MERCK) and run in chloroform-ethyl acetate (70:30) to visualize the efficiency of separation of UV fluorescent substances. Each batch was repeated several times to confirm the results.

2.9. Antibacterial activity

The purified active fraction SM-Vd was dissolved in DMSO at different concentrations (25-100 μg/mL) and used to determine antibacterial activity by a modified well diffusion assay[19], against the above listed human pathogenic bacteria. For comparison, Nalidixic acid (100 μg/mL) was used as standard antibiotic and a well filled with DMSO as negative control. Mueller-Hinton Agar (Difco) was used for this study. The plates were incubated at 37 °C for 18 h. The antibacterial activity of the purified fraction was determined by measuring the respective zones of inhibition (mm).

2.10. Determination of minimal inhibitory concentration

The antibiotic susceptibility testing of the broth micro dilution was carried out by modifying the method of Al Momani *et al*[20]. A 96 well micro dilution tray was used; the active fraction was dissolved in DMSO in the concentration of 50 μg/mL and thoroughly mixed. Five different (volume) concentrations *viz*. 1 μg/mL, 2 μg/mL, 4 μg/mL, 8 μg/mL and 16 μg/mL were used to evaluate the efficiency of antibacterial activity against the clinical isolates. The wells are then inoculated with a standardized bacterial suspension 5×105 CFU/mL using disposable device that transfers 0.01-0.05 mL into each well of the micro dilution tray. Wells containing broth alone was used as control. After 16-20 h of incubation, MICs are determined by examining the tubes macroscopically for visible evidence of bacterial growth in the form of turbidity. Wells containing the least concentration of metabolite showing no visible sign of growth was taken as the minimum inhibitory concentration.

2.11. Brine shrimp lethality bioassay

Cytotoxic activity of the purified metabolite was evaluated by using brine shrimp (*Artemia salina*)[21]. The eggs were hatched in seawater. A constant oxygen supply was maintained throughout the process. Mature nauplii were used for this study. The metabolite was dissolved in DMSO (10 mg/mL) was applied to the nauplii in each vial. However, not more than 50 μL of DMSO was added to the vials containing the brine shrimp. For each concentration, a vial containing the same volume of DMSO plus seawater and brine shrimp was used as control. The number of survivors was counted after 24 h. Based on these data, percentage of lethality of the brine shrimp nauplii was calculated. From this value, the LC_{50} of the sample was determined.

3. Result

In the course of our screening program for antagonistic actinobacteria, we isolated 46 marine actinobacterial strains. The strains were screened for antibacterial activity. From the preliminary screenings, 23 isolates showed antagonistic activity against human pathogenic bacteria. Of the 23 isolates, strain CAS72 showed higher inhibition potential against human pathogenic bacteria. The potential antagonistic actinobacteria was identified further. It was studied for physiological and morphological characteristics (Table 1). In general, aerial mycelium was red; Sporophores were spirales (Figure 1). Conidia were oblong, the surface of which was smooth (Figure 2). The chemotaxonomy of the strain CAS72 showed LL-diaminopimelic acid with glycine in their cell wall and no characteristic sugar pattern was observed. Hence, the strain belongs to the cell wall type-I. Based on these observations, the strain was grouped in to *Streptomyces* genus. Further, the strain was analyzed for its 16S rRNA gene, sequenced and identified as *Streptomyces sp.* CAS72 (Accession number: JQ425073).

Table 1
Phenotypic characteristics of the *Streptomyces sp.* CAS72.

Characteristics	*Streptomyces sp.* CAS72
Growth on ISP 2	Good
Growth on ISP 4	Good
Growth on ISP 7	Poor
Aerial spore mass colour	Red
Spore chain	Spirales
Spore surface	Smooth
Melaniod pigment	+
Reverse side pigment	Reddish orange
Soluble pigment	Violet
Growth on sole carbon sources	
D-Glucose	+
L-Arabinose	+
D-Xylose	+
Inositol	+
D-Mannitol	+
D-Fructose	+
Rhamnose	+
Sucrose	+
Raffinose	+

+ denotes presence and positive utilization, ISP: International *Streptomyces* Project

Figure 1. Spirales spore chains of the actinobacteria *Streptomyces sp.* CAS72 using light microscopy at 100×.

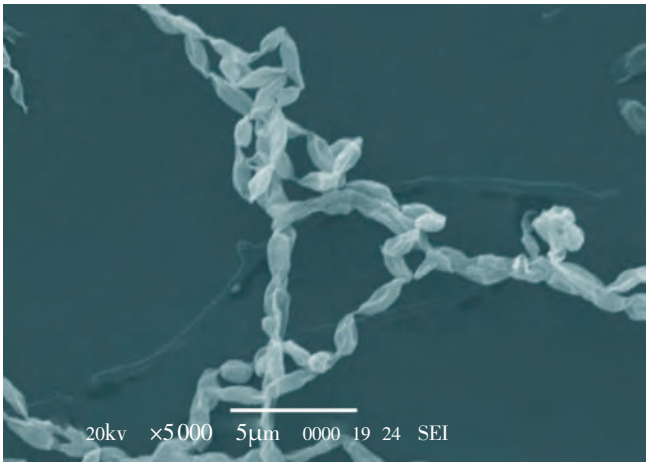

Figure 2. Smooth surface of spirales spores of the *Streptomyces sp.* CAS72 viewed under Scanning Electron Microscopy at 5000×.

The *Streptomyces sp.* was further studied for fermentation and extraction of bioactive metabolites. The SGG medium was found to be appropriate for production of novel bioactive metabolites. Post fermentation, the metabolites were extracted by liquid-liquid extraction and purified by silica gel column chromatography. A 23.8 mg of metabolite was obtained from 100 mL of the culture filtrate. The purified antibacterial metabolite with R_f value 0.74 (silica gel GF_{254}, solvent system, chloroform: ethyl acetate (5:1) was identified from ethyl acetate extract of the metabolites as reddish crystals with a melting point of 110-120 °C. The metabolite was odorless and appeared as a pinkish brown spot on the TLC plate. The metabolite showed the maximum absorption peak at the wavelength, 249 nm.

The purified metabolite was tested for antibacterial activity with different concentrations in well diffusion assay. The concentration with antibacterial activity was compared with a standard antibiotic Nalidixic acid (Table 2). The maximum zone of inhibition was found to be 8 ±0.2 (100 μg/mL) for *Klebsiella pneumoniae*-MTCC 4030 (Figure 3), 5±0.1 (100 μg/mL) for *Staphylococcus aureus*-MTCC 3160 (Figure 4), 4.5±0.1 (100 μg/mL) for *Listeria monocytogenes*-MTCC 839 (Figure 5). Whereas, the ampicillin showed 11±0.1 as maximum inhibition. When compared to standard antibiotic our purified metabolite produced relatively higher inhibition (Figure 3).Minimum inhibitory concentration of the purified metabolite was also analyzed (Table 3). The minimum inhibitory concentration (MIC) values indicated that the purified metabolite has shown less antibacterial potency, when compared to the standard antibiotic (ampicillin) tested, as revealed by minimum inhibitory concentration. For purified metabolite, the MIC values were 19, 10, 12, 10, 8, 13, 11, 13, 10, 12, 16, 11, 13 and 10 μg/mL, respectively for Escherichia coli-MTCC 1610, *Klebsiella pneumoniae*-MTCC 4030, *Listeria monocytogenes*-MTCC 839, *Salmonella enterica*-MTCC 9844, *Shigella flexneri*-MTCC 1457, *Staphylococcus aureus*-MTCC 3160, *Staphylococcus haemolyticus*-MTCC 3383, *Streptococcus pneumoniae*-MTCC 1935, *Vibrio cholerae*-MTCC 3904 and *Vibrio vulnificus*-MTCC 1145. Whereas the standard antibiotic showed MIC values between 15-22 μg/mL for different human pathogenic bacteria tested.

Table 2

Antibacterial activity of the purified metabolite and nalidixic acid against human pathogenic bacteria (Zone of inhibition mm).

	Commercial antibiotic (Nalidixic acid)		Concentration of purified metabolite (μg/mL)			
Pathogens	N	P (100 μg/mL)	25	50	75	100
MTCC 1610	–	5±0.1	Nil	Nil	Nil	1±0.1
MTCC 4030	–	10±0.1	1.5±0.1	3.5±0.1	4±0.1	8±0.2*
MTCC 839	–	10±0.1	2±0.1	2±0.1	3±0.1	4.5±0.1*
MTCC 9844	–	6±0.1	Nil	Nil	Nil	1.5±0.1
MTCC 1457	–	5±0.2	Nil	Nil	1.5±0.1	2±0.1
MTCC 3160	–	11±0.1	1±0.1	2.5±0.1	4±0.1	5±0.1*
MTCC 3383	–	10±0.1	1±0.1	1.5±0.1	2±0.1	3.5±0.1
MTCC 1935	–	8±0.1	Nil	Nil	Nil	Nil
MTCC 3904	–	10±0.1	Nil	Nil	1±0.1	2±0.1
MTCC 1145	–	10±0.1	Nil	Nil	1.5±0.1	2±0.1

*The results of *Klebsiella pneumoniae*, *Staphylococcus aureus* is significant at 100 (μg/mL) purified metabolite *vs* nalidixic acid ($P<0.05$) One-way ANOVA by Dunnett's test.
N: Nalidixic acid, P: purified metabolite.

Table 3

Minimum inhibitory concentration of the purified metabolite and standard antibiotic (nalidixic acid) against selected human pathogenic bacteria.

Pathogens	Purified metabolite (μg/mL)	Nalidixic acid (μg/mL)
MTCC 1610	19	22
MTCC 4030	10	15
MTCC 839	12	16
MTCC 9844	10	18
MTCC 1457	8	15
MTCC 3160	13	18
MTCC 3383	11	16
MTCC 1935	13	17
MTCC 3904	10	19
MTCC 1145	12	17

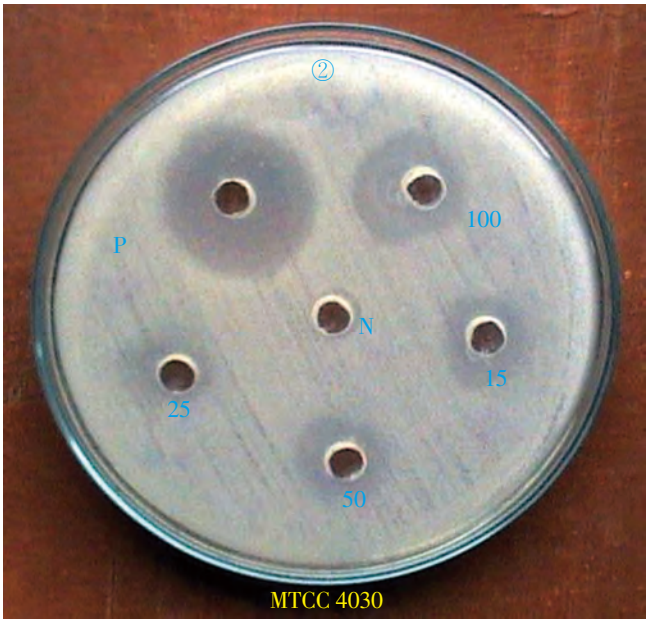

Figure 3. Antibacterial activity of the purified metabolite and Nalidixic acid against MTCC 4030. (Y ZOI 8±0.2 at 100 μg/mL) P- Positive control, N- Negative control, 25-25 μg/mL, 50-50 μg/mL, 75-75 μg/mL, 100-100 μg/mL.

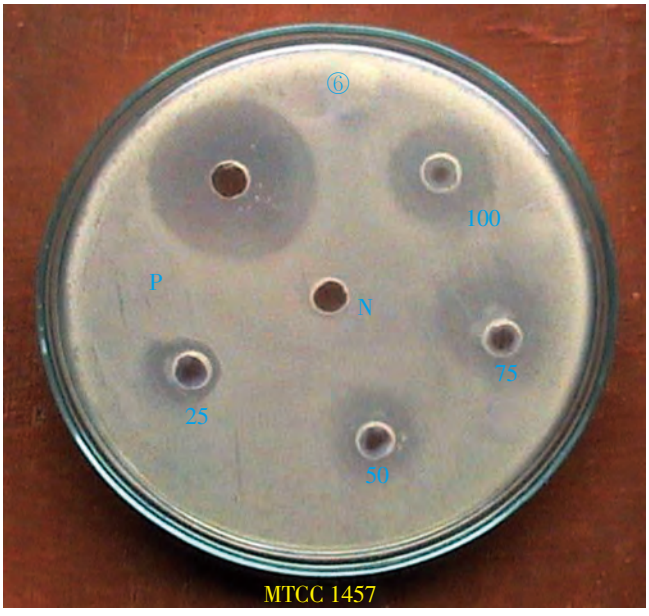

Figure 5. Antibacterial activity of the purified metabolite and Nalidixic acid against MTCC 4030. (ZOI 4.5±0.1 at 100 μg/mL) P- Positive control, N- Negative control, 25-25 μg/mL, 50-50 μg/mL, 75-75 μg/mL, 100-100 μg/mL

In brine shrimp lethality bioassay, mortality rate of the brine shrimp nauplii was found to increase with the increasing concentration of the sample and a plot concentration versus percent morality on graph showed an almost linear correlation. The metabolite, also subjected to the brine shrimp lethality bioassay for probable cytotoxic activity. The metabolite demonstrated a strong cytotoxic activity with a LC_{50} value of 23.5 μg/mL (Table 4).

Figure 4. Antibacterial activity of the purified metabolite and Nalidixic acid against MTCC 1457. (ZOI 5±0.1 at 100 μg/mL) P- Positive control, N- Negative control, 25-25 μg/mL, 50-50 μg/mL, 75-75 μg/mL, 100-100 μg/mL.

Table 4
Cytotoxic effect of the purified metabolite on brine shrimp nauplii.

Concentration of the metabolite (μg/mL)	Log concentration	% Mortality	LC_{50} value (μg/mL)
0	0	0	
10	1.0	34	
25	1.4	55	23.5
50	1.7	85	
100	2.0	100	
200	2.3	100	

4. Discussion

Marine environmental conditions are extremely different from the terrestrial environment. It infers that the marine microorganisms are different in their characteristics from those that of their terrestrial counterparts. Therefore, the marine actinobacteria are producing novel bioactive metabolites. Based on this preliminary evaluation and screening the antibacterial activity of the purified metabolite showed that it had some degree of antibacterial activity against human pathogenic bacteria. In an earlier study, Oskay *et al*. reported 32 mm (50 μg/disc) inhibition zone against *Klebsiella pneumoniae*[22]. Singh *et al.* reports that the extract of *Streptomyces* tanashiensis strain A2D showed antibacterial activity against *Bacillus subtilis* (15 mm), *Staphylococcus aureus* (25 mm), *Escherichia coli* (21 mm) and *Klebsiella pneumoniae* (23 mm)[23]. The observed minimal inhibitory concentration of the purified metabolite against the human pathogenic bacteria showed that it has less antibacterial and antifungal potency.

However, performance of the present purified metabolite is better than that of some other sources: 0.375-3 mg/mL in *Streptomyces* sp.[24] and 16-64 mg/mL of chromium based chemical complex[25]. The brine shrimp lethality bioassay for probable cytotoxic activity demonstrates that a strong cytotoxic activity with a LC_{50} value of 23.5 μg/mL. Ruhul Amin *et al*. have reported 17.78 μg/mL as LC_{50} value of penicillin extract, against the brine shrimp mortality, which is quite lower[26]. Ullah *et al*. also reported LC_{50} value of a plant extract as 8.447 to 60.323 μg/mL, which is very lower than our result[21]. Therefore, the purified metabolite can be as antitumor compound.

The search for novel metabolites especially from the marine actinobacteria requires a large number of isolates (over thousands) in order to discover a novel compound of pharmaceutical interest. The present investigation reveals the efficacy of the metabolite produced by *Streptomyces sp*. CAS72 as a bioactive compound against a variety of opportunistic human pathogenic bacteria. The strong cytotoxic activity also reveals the potentiality of the marine actinobacteria towards antitumor drug development. *Streptomyces sp*. CAS72, However, needs to be improved in their genetic level to synthesize/produce more bioactive potentials. Actinobacteria are metabolic cell factories of the marine environment. In this context, current study focused on biologically active molecules of marine actinobacteria and its availability in Uppanar estuary. Moreover, further studies need to explore such untapped resources.

Conflict of interest statement

The authors hereby declare that we have no conflict of interest whatsoever.

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Comments

Background

Marine microbiology is developing strongly in several countries with a distinct focus on bioactive compounds. Actinobacteria are well known as secondary metabolite producers and hence of high pharmacological and commercial interest. Naturally occurring about 23,000 antibiotics have been discovered from microorganisms. It has been estimated that approximately 10 000 of them were isolated from actinomycetes. Actinomycetes, mainly the genus *Streptomyces*, have the ability to produce a wide variety of secondary metabolites as bioactive compounds, including antibiotics. The genus *Streptomyces* is represented in nature by the largest number of species among all the genera of actinomycetes and figures over 500 species. Actinomycetes comprise about 9% of the bacteria colonising marine aggregates and can be isolated from various marine sources. Many actinomycete isolates from the depths of the oceans contain non-ribosomal polyketide synthase and polyketide synthase pathways, the hallmarks of secondary metabolite production.

Research frontiers

Studies are performed to determine the significant anti microbial effects from the isolated and characterized actinobacteria of the genus *Streptomyces*. The samples were collected from Cuddalore estuary in India.

Related reports

Abdelmohsen *et al*. (2010) isolated 90 actinomycetes from 11 different species of marine sponges that had been collected from offshore Ras Mohamed (Egypt) and anti-infective activities was performed against clinically relevant, Gram-positive (*Enterococcus faecalis*, *Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria, fungi (*Candida albicans*) and human parasites (*Leishmania major*, *Trypanosoma brucei*). Uthiraselvam *et al*. (2011) isolated 17 actinomycetes from leaves of 5 different halophytic plants such as *Avicennia marina*, *Bruguiera cylindrica*, *Rhizophora mucronata*, *Salicornia brachiata* and *Suaeda monoica* and tested for antibacterial activity against some bacterial pathogens. Valli *et al*. (2012) isolated 21 *Actinomycetes* species from marine environment and tested from antibacterial activity against pathogenic bacteria.

Innovations & breakthroughs

Data regarding this study Actinobacteria was isolated from the sediment of Uppanar estuary, Cuddalore, India. The isolated actinobacterial strains were analyzed antimicrobial screening. Marine actinobacteria are the most economically as well as biotechnologically valuable prokaryotes. This paper focused on specific topics and can be of high interest to the readers.

Applications

Marine actinobacteria have proven to be efficient producers of bioactive metabolites which have a wide range of activities such as antibacterial, antifungal, antitumour, anticancer, cytotoxic, *etc*. This study may be useful for antimicrobial actions.

Peer review

This is a good study in which authors focused on specific area. Marine actinobacteria was isolated from estuary. From this 46 marine actinobacterial strains was isolated and screened 23 isolates. Then secondary metabolites were isolated, characterized, purified and checked the different antimicrobial activity compared with standard antibiotic nalidixic acid.

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