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ANTIBACTERIAL ACTIVITY OF *STREPTOMYCES* SP SH7 ISOLATED FROM CARDAMOM FIELDS OF WESTERN GHATS IN SOUTH INDIA

D. GEETHA RAMANI*[1] AND T. VINOTH KUMAR[2]

[1] Department of Microbiology, Dr. N. G. P. Arts and Science College, Coimbatore, Tamil Nadu, India
[2] Department of Biotechnology, C. M. S. College of Science and Commerce, Coimbatore, Tamil Nadu, India

ABSTRACT

One hundred and forty seven strains of actinomycetes were isolated from soil samples collected from cardamom fields at Western Ghats of South India. The antimicrobial activities of the isolated strains were studied by well diffusion method. Eighty-eight isolates exhibited antibacterial activity against the target test bacteria. The isolate *Streptomyces* sp Sh7 exhibited promising antibacterial activity against Gram positive and Gram-negative bacteria. Identification of the isolate up to genus level was done. Optimization of cultural conditions for antibiotic production by the isolate Sh7 was carried out. HPLC-DAD (High performance liquid chromatography-Diode Array Detector) analysis of the crude culture filtrate was performed to detect the presence of any known antibiotic compound. The antibiotic was found to be extracellular in nature. The culture filtrate was subjected to sequential solvent extraction. The antibacterial compounds were extracted using butanol and purified by Thin Layer Chromatography. The maximum absorption spectra of the purified antibacterial compounds were determined.

KEY WORDS : ***Antimicrobial activity, Actinomycetes, TLC, HPLC-DAD, Maximum absorption spectra***

D. GEETHA RAMANI
Department of Microbiology, Dr. N. G. P. Arts and Science College,
Coimbatore, Tamil Nadu, India

INTRODUCTION

Various organisms like bacteria, fungi, higher plants, animals and actinomycetes produce antibiotics. Actinomycetes are filamentous soil bacteria, which are considered important in relation to other species because of their ability to produce antibiotics. Actinomycetes produce most of the naturally occurring antibiotics used in medicine today. Nearly 8000 antibiotics from actinomycete are described so far, of which 80% were from *Streptomyces* sp and 20% from other actinomycete genera[1]. Every antibiotic placed in use has endangered resistance irrespective of its chemical class or molecular target. As current antibiotic therapy grows increasingly ineffectual, it is critically important to isolate structurally novel antibiotics that forecast the emergence of antibiotic resistance. Thus, the development of antibiotics is ultimately a futile endeavor. *Streptomyces* sp is the largest antibiotic producing genus in the microbial world[2]. Recent reports show that this group of microorganisms remains an important source of antibiotics[3]. Intense screening of actinomycetes especially rare actinomycetes is taking place all over the world[4]. Exploration of actinomycetes diversity from cardamom fields at Western Ghats of South India has not been reported so far. Thus, the present investigation focuses at the isolation of biotechnologically significant strains of antibiotic producing soil actinomycetes from cardamom fields and for the partial purification of the antibiotic being produced.

MATERIALS AND METHODS

The solvents used for the study were of analytical grade (AR grade) and all the media ingredients and chemicals used in the investigation were purchased from Hi Media Laboratories, India.

About 25 soil samples were collected from different cardamom fields in Western Ghats of Idukki district at Kerala, South India. Each collection was made from 10-15 cm depth of the soil. About 10 grams of soil sample was mixed with one gram of calcium carbonate and incubated at 26°C for five days, to enrich the population of actinomycetes present in the soil samples[5].

(i) Isolation of actinomycetes and screening for antimicrobial activity

After pretreatment, the soil samples were plated by dilution plate technique on Starch Casein Nitrate agar (SCN) with fifty micrograms per ml of Cycloheximide[6]. The plates were then incubated at 25°C for 5 to 15 days and observed daily for the growth of actinomycetes. The isolated colonies were subcultured on SCN agar and stored in a refrigerator at 4°C.

Morphologically distinct actinomycetes isolates were subjected to fermentation for antibiotic production on Soluble Starch medium (SS medium). SS medium containing soluble starch 25g/L, glucose 10g/L, yeast extract 2g/L, calcium carbonate 3g/L and trace salt solution 1ml/L was prepared and dispensed into Erlenmeyer Flaks. The pH of the medium was adjusted to 7.3. The medium was inoculated with 1×10^6 spores/ml of the actinomycete strains. The contents of the flasks were then subjected to fermentation in an orbital shaker at 210 rev/min and room temperature for 5 days.

After 5 days of fermentation, the culture filtrate was centrifuged and separated from the cell mass. The culture filtrate was then condensed to 10ml final volume by lyophilisation. The condensed culture filtrate was subjected to screening for antimicrobial activity by well plate method[7] on Muller-Hinton agar plates (pH7) seeded with the target test bacteria namely, *Staphylococcus aureus* MTCC 96, *Escherichia coli* MTCC 739, *Klebsiella pneumonia* MTCC 3384, *Pseudomonas aeruginosa* MTCC1688 and *Bacillus cereus* MTCC 430. The Muller Hinton agar plates were incubated at 37°C for 24 hours and observed for antibacterial activity of the culture filtrate on test bacteria. Among the isolates screened, the antimicrobial activity of strain Sh 7 was promising against the test bacteria and it was selected for further study.

(ii) Detection of Antimicrobial Activity in the Cell mass and Supernatant

To test, if the antimicrobial metabolite was extracellular or intracellular, the culture filtrate of Sh7 was centrifuged at 10,000 rev/min for 30 minutes. The ethanol extract of the dried mass was tested for antibacterial activity after removing the solvent ethanol *in vaccuo*.

(iii)Characterization of selected actinomycete isolate

The growth of the isolate Sh7 was studied at 22°C, 28°C, 37°C and 42°C temperatures. The strain was subjected to cultural, morphological and biochemical characterization[8].Surface and aerial mycelia were observed by agar block method. Cultural characterization was done on International Streptomyces Project Media (ISP medium) at 37°C. Utilization of different carbon sources and nitrogen sources were studied. The cells were analyzed for 2, 6, Diaminopimelic acid[9] and whole cell sugar content[10]. Enzyme activity was performed on egg yolk medium[11] and chitin agar[12]. Degradation of starch (1% w/v), gelatin (0.4% w/v), urea (1% w/v), casein (1% w/v), xanthine (0.4% w/v), hypoxanthine (0.4% w/v) and adenine (0.5% w/v) was performed on modified Bennett's agar. DNase activity was assayed on DNase test agar[13]. Growth in the presence of chemical inhibitors like crystal violet (0.0001% w/v), phenol (0.1% w/v) and sodium chloride (4, 7, 10, 13% w/v) was examined on modified Bennett's agar. Presence or absence of growth on media with inhibitors was noted after 7 and 14 days respectively.

(iv)Optimization of cultural conditions for antibiotic production by the isolate Sh7:

Days of Fermentation

SS broth was prepared in 12 separate flasks and inoculated with $1x10^6$/ml of spores of the isolate Sh7. The flasks were incubated at room temperature for 12 days at 210 rev/min. After every 24 hrs, one flask was removed from the shaker. The culture filtrate was separated from the mycelium by centrifugation at 10,000 rev/min for 30 minutes. The separated culture filtrate was then condensed to a final volume of 10ml by lyophilisation. Antibiotic production by the isolate was monitored by well diffusion method after every 24 hours and biomass was determined in terms of cell dry weight of the mycelium from 100 ml of the broth after drying at 60°C until a constant weight is reached.

pH

The effect of pH on antibiotic production was determined by subjecting the isolate Sh7 for fermentation in SS medium, separately maintained at different pH values of 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7 and 7.8. pH was adjusted with 0.1N sodium hydroxide and 0.1N Hydrochloric acid[14]. All the flasks were inoculated with 1×10^6 spores/ml of the isolate Sh7 and incubated at room temperature in an orbital shaker at 210 rev/min for 8 days.

Temperature

Five Erlenmeyer flasks with SS broth were inoculated with $1x10^6$/ml of spores of the isolate Sh7. The flasks were incubated at 24°C, 28°C, 32°C, 37°C and 42°C in an orbital shaker at 210 rev/min for 8 days.

Agitation

Five Erlenmeyer flask with SS broth was inoculated with $1x10^6$/ml of spores of the isolate Sh7. The flasks were incubated in an orbital shaker at 190, 200, 210, 220, 230 rev/min for 8 days.

Size of the inoculum

Streptomyces sp Sh7 was inoculated onto SCN slants and incubated at 28°C for 5 days. After five days of incubation, the spores were skimmed off from the surface of the slants with 3 ml of physiological solution containing 0.1 % v/v Tween 80. The spore concentration in the suspension was quantified by standard viable plate count method. The suspension was used to prepare spore suspensions ranging in concentration from 1×10^8 to 6×10^8 CFU/ml. SS broth was prepared in six different Erlenmeyer flasks, inoculated with 0.5 ml spore suspensions of *Streptomyces* sp Sh7 ranging in concentration from 1×10^8 to 6×10^8 CFU/ml and subjected to fermentation at

room temperature in an orbital shaker at 210 rev/min for 8 days.

After 12 days of fermentation, in case of all the above-mentioned experiments, the culture filtrate was centrifuged at 10,000 rev/min for 30 minutes and separated from the cell mass. The culture filtrate was then condensed to 10ml final volume by lyophilisation. The condensed culture filtrate was subjected to screening for antibacterial activity against *S. aureus* MTCC 96 and *E. coli* MTCC 739 by well plate method on Muller–Hinton agar plates (pH 7) seeded with the test bacteria.

(v) Identification of the presence of known antibiotic produced by the isolate Sh7

To identify the presence of known antibiotics produced by the isolate Sh7, the condensed crude culture filtrate of the isolate Sh7, after 8 days of fermentation, was subjected to HPLC-DAD screening. To identify the presence of known antibiotics produced by the isolate Sh7, the HPLC-DAD screening was performed. After 8 days of fermentation, the Methanol-Acetone (1:1) extract and water fraction of the condensed crude fermentation broth were analyzed by gradient reversed phase HPLC coupled with a Diode array monitoring system. The HPLC conditions were as follows: The chromatographic system consisted of HP 1090M liquid chromatography equipped with diode array detection system.

Stainless steel column of Nucleosil-100 C-18 (5µm), 4.6 mm I.D. x 125 mm with 20 mm guard column was used. Ten microliter of the sample was injected on to the guard column. The samples were analyzed by linear gradient elution using 0.1% o-phosphoric acid as solvent-A and Acetonitrile as Solvent-B at a flow rate of 2ml/min. The gradient was from 0 to 100% B within 15 minutes with a 2-minutes hold at 100% B, followed by a 5 min post-time at 100% A. multiple wavelength monitoring was performed at DAD wavelengths 210, 230, 260, 280, 310, 360, 435, and 500 nm. The resultant peaks were compared by their UV-Visible spectra with those of more than 600 reference compounds mostly antibiotics, stored in the database.

Sequential Solvent Extraction of the Antibiotic Produced by the Strain

About 50ml of the condensed crude culture filtrate of the isolate Sh7 was subjected to sequential solvent extraction in a separating funnel using solvents of varying polarity namely n-hexane, chloroform and n-butanol. The extracts were then dried *in vacuo* to remove the solvents used for extraction. The components extracted were reconstituted in water and 50µl of the extract was subjected to antibacterial activity against the test bacteria by well diffusion assay.

(vi) Separation and Purification of Antibiotic by Thin Layer Chromatography(TLC)

To separate and purify the antibiotic produced, the butanol extract, which showed activity on well plating, was subjected to TLC employing various solvent systems (Table I). The silica gel G60 plate (Plate dimension 20×5 cm and a layer thickness of 0.25mm) were prepared and activated at 110°C for two hrs. The butanol extract suspended in water was then spotted on the TLC plate using a capillary tube. The plates were then developed in closed glass chambers saturated with the developing solvents. After a plate had run, it was removed from the developing chamber and dried in air. The separated compounds were then visualized by a non-destructive procedure using ultraviolet light. The components separated were then scrapped from the plates and collected in Eppendoff® microfuge tubes.

(vii) Determination of Antibiotic Activity of the Compounds Separated by Thin Layer Chromatography

The compounds collected in eppendoff tubes were then extracted with butanol. Butanol extract was then dried *in vacuo* and 50µg of the extracted compound was reconstituted in 50µl of water. Fifty microlitre of the suspension was then loaded in to the wells on Muller-Hinton agar seeded with the bacterial test culture. The plates were incubated at 37°C overnight to determine the

antibacterial activity of the separated compounds.

(viii) Determination of Maximum Absorption Spectra of the Antibiotic Compounds Separated by Thin Layer Chromatography:

The maximum absorption spectra of the antibiotic components separated by TLC were determined in a Thermo Spectronic® genesis 10 UV spectrophotometer. A full range scanning was performed (190-1100nm). A base line scanning was collected by using the solvent blank (MilliQ® Ultra pure water).

RESULTS

1. Isolation and Screening for Antimicrobial Activity

About 147 strains of actinomycetes were isolated and screened for antibacterial activity against a panel of Gram negative and Gram-positive bacteria (data not shown). Among them, 88 isolates exhibited antibacterial activity against the test bacteria. Among the 88 isolates, the antibacterial activity of the isolate Sh7 was promising against both Gram positive and Gram negative test bacteria (Table II). The isolate was selected for further study.

2. Optimization of Cultural Conditions for Antibiotic Production

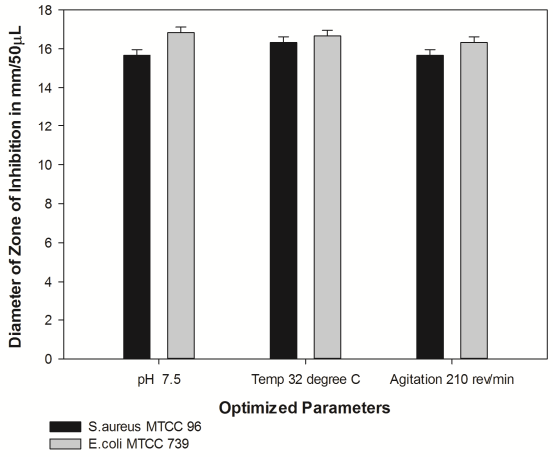
The course of antibiotic production and the optimum cultural conditions for antibiotic production by the isolate *Streptomyces* sp Sh7 are shown in Fig 1a & 1b respectively. Antibiotic activity was observed from the 4th day of fermentation but the maximum activity reached on the 8th day of fermentation. After the 8th day of fermentation, no further increase in antibiotic activity was observed and the antibiotic activity was observed until the 12th day of fermentation without any cessation.

The optimum pH for antibiotic production by *Streptomyces* SH7 was found to be 7.5. The optimum temperature for antibiotic production was found to be 32°C. Deviation from this affects the production of antibiotic. Agitation affects aeration and mixing of nutrients in the fermentation medium. The agitation rate of 210 rev/min was found to be optimum for antibiotic production by *Streptomyces* sp SH 7. No antibiotic production was observed in the absence of agitation.

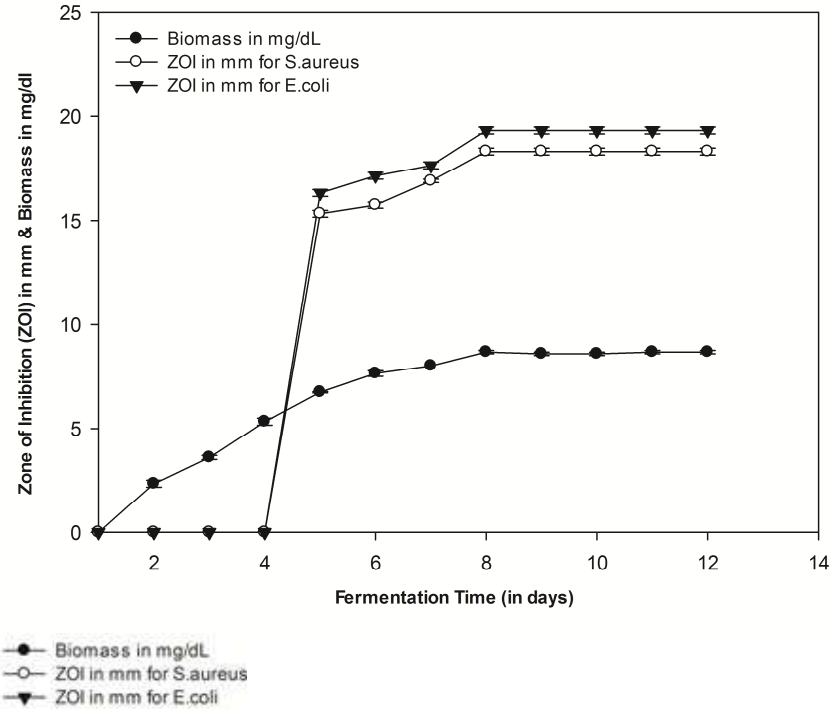
1a: Optimization of cultural conditions for antibiotic production by S*teptomyces* sp SH7

Graph 1a

Optimum cultural conditions for antibiotic production by Steptomyces sp SH7



Graph 1b
Course of antibiotic production by submerged fermentation (in days) by Steptomyces sp SH7



Biomass in mg/dL
ZOI in mm for S.aureus
ZOI in mm for E.coli

3. Characterization of actinomycete Isolate

The selected isolate Sh7, exhibited optimal growth at 37°C. The colony morphology on ISP media 1-7 showed grey aerial mycelium and yellow color on the reverse side. No soluble pigment production was observed. The presence of LL-DAP and the absence of characteristic sugars in whole-cell hydrolysates showed that strain Sh7 had wall type I[8]. The biochemical characteristics are tabulated in Table 1. The taxonomic properties described above apparently suggested that the isolate Sh-7 belongs to the Genus *Streptomyces* and designated as *Streptomyces* sp Sh7.

Table 1
Biochemical Characteristics of Streptomyces sp strain SH7

ISP Medium 1-7	Cream-aerial mycelium Yellow-reverse
Starch Agar	+ve Hydrolysis
Skim Milk Agar	+ve Hydrolysis
Gelatin Hydrolysis	-ve Hydrolysis
Urea Hydrolysis	+ve Hydrolysis
Xanthine Agar	-ve Hydrolysis
Hypoxanthine Agar	-ve Hydrolysis
Resistance to Crystal violet Phenol: NaCl 4% NaCl 7% NaCl 10% NaCl 13%	 No resistance

Growth in the presence of carbon source	
L-Arabinose	+ve
D-Fructose	+ve
D-Galactose	+ve
Meso-Inositol	+ve
Mannitol	+ve
Adonitol	-ve
Rhamnose	+ve
Xylose	-ve
Salicin	-ve
Sucrose	-ve
Growth in the presence of nitrogen source	
L Alanine	+ve
L-Cysteine	+ve
L-Histidine	-ve
L-Leucine	+ve
L-Phenylalanine	-ve
L-Valine	-ve
DNase activity	+ve
Modified egg yolk agar	-ve

+ve : positive ; -ve: negative

Detection of Antimicrobial Activity in the Cell Mass and Supernatant

The ethanol extract of the cell biomass did not show any antimicrobial activity. However, the culture supernatant inhibited all the test bacteria/ microorganisms (Table 2). This clearly shows that the antibiotic produced by the isolate Sh7 is extracellular in nature.

Table 2

Antimicrobial Activity of the condensed crude culture filtrate (50µl) of Streptomyces sp Sh7

Target/ Test bacteria	**Diameter of zone of inhibition in mm***
S. aureus MTCC 96	15.33±0.33
E. coli MTCC 739	16.67±0.33
K. pneumoniae MTCC 3384	15.67±0.33
P. aeruginosa MTCC1688	12.33±0.33
B. cereus MTCC 430	17±0.00

****All the values are mean ± SEM of three determinations.***

HPLC-DAD Analysis

The methanol-acetone extract and water extract of the fermentation broth, upon subjecting to HPLC-DAD detection resulted in numerous peaks (Fig 2). None of the resultant peaks showed conformity with the reference compounds in the database. HPLC-DAD analysis reveals that the antibiotic produced do not show conformity with the spectral data of any of the known antibiotics available in the database[15].

HPLC DAD Analysis

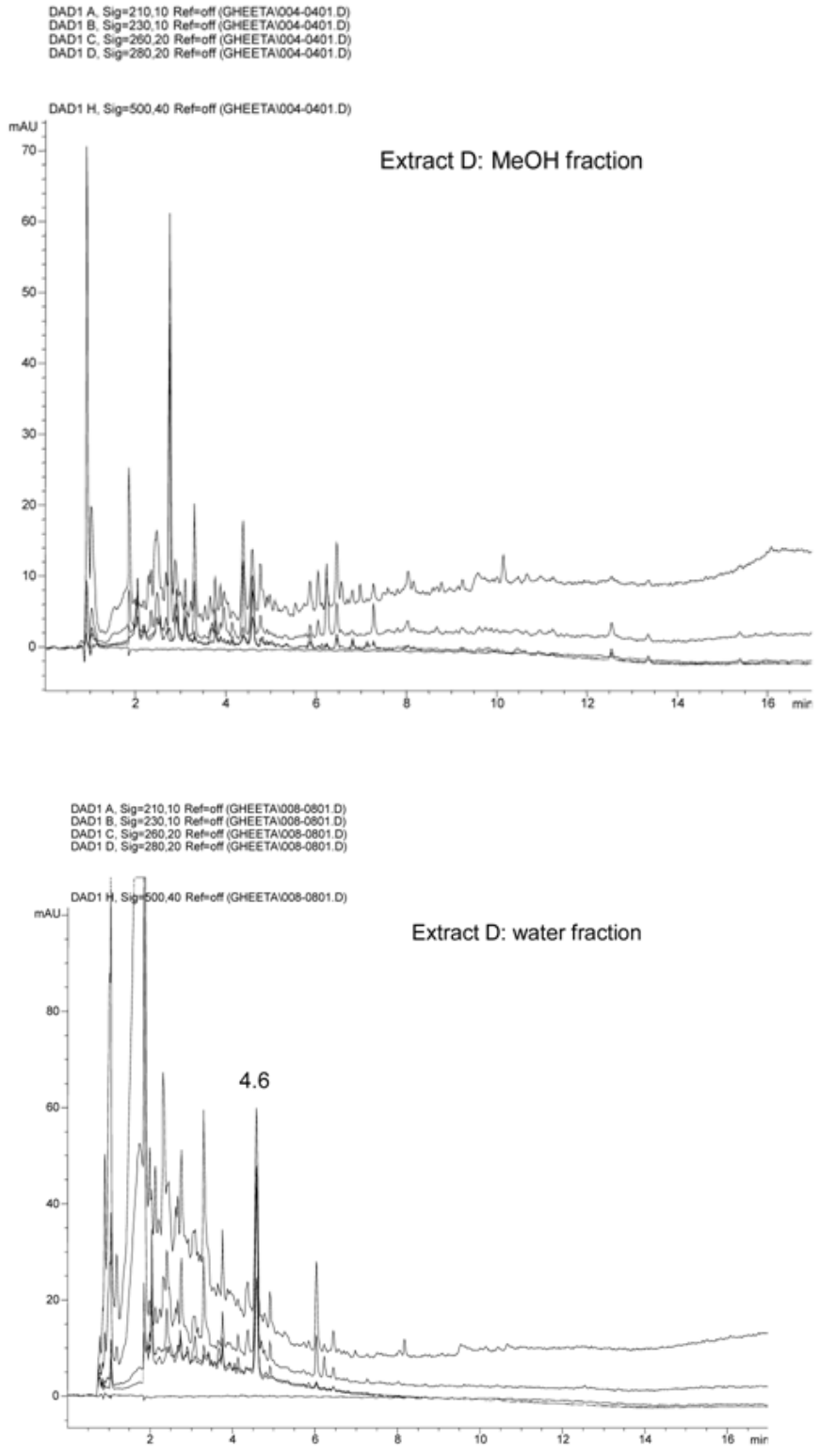


Figure 2
HPLC DAD Analysis of Active Fractions

Extraction, Separation and Purification of the Antibiotic Produced by the Strain Sh7

The antibiotic components were extracted in solvent butanol. The extracted compounds were then separated as discrete spots on TLC in solvent system H (Table 3). Two spots corresponding to Rf value of 0.68 (compound A) and 0.92 (compound B) were found to possess antibacterial activity against Gram positive and Gram-negative bacteria respectively (Table 4).

Table 3
Developing Solvent System for purification and separation of antibiotic by TLC

System	Developing system	Composition v:v
A	Acetone: Methanol	50:50
B	Isopropanol: Methanol	30:70
C	Butanol: Methanol: Water	35:30:35
D	Butanol: Methanol: Water	35:35:30
E	Butanol: Metanol: Water	45:40:20
F	Butanol: Acetic Acid: Water	40:10:50
G	Butanol: n-Propanol: Water	50:40:10
H	Butanol: n-Propanol: Water	40:40:20
I	Butanol: n-Propanol: Water	45:45:10

Table 4
Antibacterial activity of compound A and compound B purified by TLC

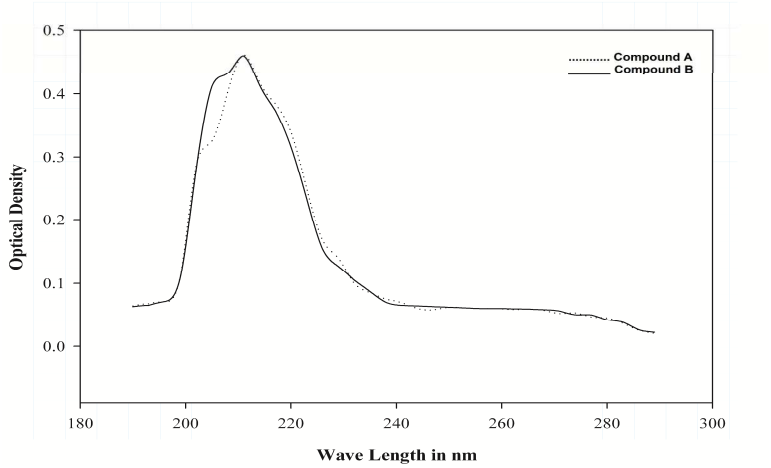
Target /Test bacteria	Diameter of zone of inhibition (in mm) *	
	Compound A (50µg)	Compound B (50µg)
S. aureus MTCC 96	16.67± 0.33	-
E. coli MTCC 739	-	16.33±0.88
K. pneumoniae MTCC 3384	-	15.67±0.33
P. aeruginosa MTCC1688	-	13.67±0.33
B. cereus MTCC 430	17.67± 0.88	-

All the values are mean±SEM of three determinations; '-' No significant zone of inhibition

Maximum Absorption Spectra of the Antibiotic Produced.

The maximum absorption spectra of both compound A and compound B were found to be 211 nm (Fig 3). It has been reported previously that a strain of S*treptomyces* sp produced an antibiotic compound with absorption spectrum of 211 nm [16].

Figure 3
Maximum Absorption Spectra of Compound A & B



— Compound A
… Compound B

DISCUSSION

In the present study, among the 147 actinomycete isolates screened for antibacterial activity, 60% of isolates exhibited antibacterial activity against the test bacteria. Actinomycetes have been recognized as the potential producers of antibiotics. Among the various genera of actinomycetes, the Streptomyces genus is a remarkably rich source of natural products, accounting for the production of two-thirds of commercially available antibiotics[17.] It is noteworthy that this genus still produces a larger number and a wider variety of new antibiotics than members of any other genus and seems to be an almost inexhaustible reservoir of novel antibiotics[18]. Western Ghats, being an important hot spot for biodiversity have not been much explored for its microbial populations. The present study aimed at isolating potent actinomycetes with potent antimicrobial capacity from cardamom fields of Western Ghats in South India. The study was successful isolating a potent *Streptomyces* species designated *Streptomyces* sp SH7.

Production of antibiotics has been known to be influenced by media components and cultural conditions such as pH, temperature, days of fermentation and aeration[19]. *Streptomyces* sp Sh 7 produces antibiotic from the 4th day of growth and antibiotic production continued without any reduction until the 12th day of fermentation. This is because of the fact that antibiotics are secondary metabolites that are produced at the end of the growth phase[20]. Microorganisms vary in their cultural conditions and optimal parameters for antibiotic production. Among them, temperature, pH, nutrients, agitation, and aeration are the most typical factors. These factors influence bacterial growth thereby affect antibiotic production [21, 22]. It has been reported that the change in pH of the culture medium induces production of new products that adversely affects antibiotic production [23.] There will be better transfer of oxygen and enhanced uptake of nutrients during agitation. Microorganisms show variation in their temperature requirement for antibiotic production. Cryomycin a peptide antibiotic is produced by a strain of *Streptomyces* only at low temperature [24]. The antibiotic produced by *Streptomyces* SH7 is extracellular in nature as in most of the cases; antibiotics are extracellular[25].

CONCLUSION

The present study was successful in isolating a potent *Streptomyces* species from Western Ghats. As indicated by the results, the antibiotics produced by the organism could be novel. Confirmations of novelty of the compounds need further investigations to determine the functional group, elucidate the structure and physicochemical nature of the antibiotic. Further studies are under progress for molecular characterization of the isolate, to determine the structure, nature of the antibiotics, for industrial scale production and its application for human welfare. It is concluded from the present study that, the actinomycetes, even today, are a source for new antibacterial antibiotics.

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REFERENCES

1. Hu H, Ochi K, Novel approach for improving the productivity of antibiotic-producing strains by inducing combined resistant mutations. Appl Environ Microbiol, 67:1885-92, (2001).
2. Korn-Weindisch F and Kutzner H J. The family *Streptomycetaceae*: In: A Balows, HG Truper, M Dworkin, W Harder, KH Schleifer (eds.), The prokaryotes, vol. 1, 2nd edn, Springer-Verlag, Berlin, 1992, pp 921-995.
3. Watve MG, Tickoo R, Jog MM, Bhole BD, How many antibiotics are produced by the genus Streptomyces? Arch Microbiol, 176: 386-390, (2001).
4. Bull AT, Ward AC, Goodfellow M, Search and discovery strategies for biotechnology: The paradigm shift. Microbiol. Mol. Biol. Rev, 64: 573-606, (2000).
5. Saadoun I, Gharaibeh R, The *Streptomyces* flora of Badia region of Jordan and its potential as a source of antibiotics active against antibiotic-resistant bacteria. J Arid Environ, 53: 365-371, (2003).
6. Abdelghani Zitouni, Hadjira Boudjella, Lynda Lamari, Boubekeur Badji, Florence Mathieu, Ahmed Lebrihi, Nasserdine Sabaou, Nocardiopsis and Saccharothrix genera in Saharan soils in Algeria: Isolation, biological activities and partial characterization of antibiotics. Res. Microbiol, 156:984–993, (2005).
7. Augustine SK, Bhavsar SP, Kapadnis BP, A non-polyene antifungal antibiotic from *Streptomyces albidoflavus* PU 23. J. Biol. sci, 30: 201-211, (2005).
8. Shirling B, Gottlieb D, Methods for characterization of *Streptomyces* sp. Int. J. Syst. Bacteriol, 16: 3313-3340, (1966).
9. Becker B, Lechevalier MP, Gordon RF, Lechevalier HA, Rapid differentiation between Nocardia and Streptomyces by paper chromatography of whole cell hydrolysates. Appl. Microbiol, 12: 421-423, (1964).
10. Lechevalier MP, Lechevalier HA, Chemical composition as a criterion in the classification of aerobic actinomycetes. Int. J. Syst. Bacteriol, 20: 435-443, (1970).
11. Nitsch B, Kutzner HJ, Egg yolk medium as a diagnostic medium for Streptomycetes. Experientia, 25: 220-221, (1969).
12. Hsu SC, Lokwood JL, Powdered chitin agar as a selective medium for enumeration of actinomycetes in water and soil. Appl. Microbiol, 29: 422-426, (1975).
13. Goodfellow M and Alderson G, Numerical taxonomy of Actinomadura and related Actinomycetes, J. Gen. Microbiol. 112: 95-111, (1979).
14. Maha A. Hassan, Moustafa Y, El-Naggar, Wafa Y. Said, Physiological factors affecting the production of an

antimicrobial substance by *Streptomyces violatus* in batch cultures. Proceedings of the First International Conference (Egyptian British Biological Society, EBB Soc) Egyptian Journal of Biology, 3:1-10, (2001).
15. Hacene H, Daoudi H, Bhatnagar T, Beratti JC, Lefebrre G, A new aminoglycosidase antipseudomonas antibiotic produced by a strain of *Spirillospora.* Microb, 102:69, (2000).
16. Fiedlar HP, Biosynthetic capacities of Actinomycetes, Screening of secondary metabolites by HPLC and UV Visible absorbance spectral libraries. Nat. Prod. Lett, 2:119, (1993).
17. Bentley SD, K. F. Chater, A. M. Cerdeno-Tarraga, G. L. Challis, et al Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3 (2). Nature 417:141-7, (2002).
18. Berdy, J. Bioactive microbial metabolites. J Antibiot 58:1-26, (2005).
19. Nuerittin S, Aysel U (2003) Investigation of antimicrobial activity of some *Streptomyces* isolates. Turk. J. Biol. 27:79-84
20. Iwai Y, Omura S, Cultural conditions for screening of new antibiotics. J. Antibiotic, 35:123, (1982).
21. Yoshida N, Tani Y, Ogata TC, Cryomycin a new peptide antibiotic produced only at low temperature. J. antibiot, 25: 653, (1972).
22. Akhurst RJ, Antibiotic activity of Xenorhabdus spp., bacteria symbiotically associated with insect pathogenic nematodes of the families Hettrorhabditidae and Steinernematidae. J Gen Microbiol, 128: 3061–3065, (1982).
23. Reichenbach H, Gerth K, Irschikunze HB, Hofle G, Myxobacteria: A source of new antibiotics. Trends biotech, 6:115-121, (1988).
24. Omura s, Nakagawa A, Yamada H, Hata T, Furusaki A, Watanabe, TC, Structure and biochemical properties of Kanamycin A,B,C and D. Chem pharm Bull,21:931, (1973).
25. Chen G, Maxwell P, Dunphy GB, Webster JM, Culture conditions for Xenorhabdus and Photorhabdus symbionts of entomopathogenic nematodes. Nematologica, 42:124–127, (1996).