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Research Article

STREPTOMYCES CHARTREUSIS STRAIN *ACTM-8* FROM THE SOIL OF KODAGU, KARNATAKA STATE (INDIA)*:* ISOLATION, IDENTIFICATION AND ANTIMICROBIAL ACTIVITY

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ABSTRACT

The actinomycetes strain designated as ACTM-8 isolated from unexplored soil samples of Madikeri Taluk in Kodagu district, Karnataka State (India). The strain of ACTM-8 characterized based on the polyphasic taxonomic analysis, such as cultural, morphological and biochemical characteristics as well as 16S rRNA gene sequence analysis, as *Streptomyces chartreusis* (accession number KJ024097). The strain evaluated for antimicrobial activity against test microorganisms in two steps: primary and secondary screening. It showed a broad spectrum of antimicrobial activity against both test bacteria and fungi. The ethyl acetate extract of strain ACTM-8 was the most effective against *Fusarium graminarium* (36.33 ± 0.57mm) followed by *F.poea* (33.1 ±0.11mm), *F.sporotrichioiodes* (30.26 ±0.57mm), *F.equeseti* (24.3± 0.17mm), *F.nivale* (18.33 ± 0.11mm), *Enterobacter aerogenes* (12 .16± 0.28mm), *Bacillus subtilis* (10.66± 0.57mm) and *Staphylococcus aureus* (10.33 ± 0.57 mm) respectively. Therefore, the isolate ACTM-8 represents as the potent broad spectrum antimicrobial compound , may provide a valuable resource for the development of novel compound for pharmaceutical applications.

Keywords: *Streptomyces chartreusis*; Antimicrobial activity; *Fusarium* spp.; Soil; India.

INTRODUCTION

Members of the class of Actinobacteria are filamentous bacteria which are the most beneficial sources for the production of a diverse range of bioactive metabolites viz antibiotics with high commercial value(Watve et al,2001; Berdy,2005). Currently, the results of extensive microbial screening have been discovered that about half (8,700) of the natural occurrence microbial source of antibiotics, have been produced by actinomycetes, out of which 6,500 are derived from *Streptomyces* species, which are used for pharmaceutical and agricultural applications (Berdy,2005; Tiwari and Gupta, 2012; Dezfully and Heidari, 2016).

With this perspective, each year, several investigations have been done on the screening of the new isolates of actinomycetes particularly *Streptomyces* which were able to produce antimicrobial agents around the world (Saintpierre-Bonaccio et al, 2004; Veyisoglu et al, 2014; Maciejewska et al, 2015).

Although, soil ecosystems, serve as a reservoir for actinomycetes and their bioactive metabolites, only a limited fraction of soil-derived actinomycetes have been explored till now (Rosselló-Mora and Amann,2001). Hence, there is a need for increased exploration of under and un-explored habitats for isolation of new actinomycete taxa and novel bioactive metabolites for the next generation of pharmaceutical agents.

The Kodagu district as a part of Western Ghats is one of the two important world's biodiverse hotspots located on the Indian subcontinent which, has a rich and varied heritage of biodiversity (Aravind et al, 2007). However, not much work has been done on the actinomycetes screening and their utility in the rich biodiverse region (Khandan and Janardhana, 2013; Dezfully and Ramanayaka ,2015).

Hence, the present study was carried out to isolate of the *Streptomyces* sp. strain ACTM-8 from soil samples of the Kodagu district of Karnataka, India and to evaluate its

antimicrobial activity against Gram-positive and Gram- negative bacteria and cereal pathogenic fungi and its identification by polyphasic taxonomic analysis.

MATERIALS AND METHODS

Soil collection

Soil samples were accumulated from the grasslands of Madikeri Taluk in Kodagu district, Karnataka, India. The air-dried soil samples were pretreated with 1% $CaCO_3$ to enrich the actinomycetes population (Tsao et al, 1960).

Actinomycete isolates

The serial dilution method on Actinomycete isolation agar medium was done to isolate and numeration of actinomycetes present in the soil samples (El-Nakeeb and Lechevalier, 1963). The suspected colonies were purified on Yeast Extract - Malt Extract Agar medium (ISP-2) (El-Nakeeb and Lechevalier, 1963) and was maintained at refrigerator for further analysis.

Characterization of the strain ACTM-8

The ACTM-8 strain was characterized by morphological, cultural and biochemical physiological parameters as well as 16S rRNA gene sequence analysis.

Cultural and morphological parameters

The cultural characteristics of strain ACTM-8 was studied on various media viz; Tryptone Yeast Extract Agar (ISP-1), ISP-2, Oat Meal Agar (ISP-3), Inorganic Salt Starch Agar (ISP-4), Glycerol -Asparagine Agar (ISP-5), Peptone Yeast Extract Iron Agar (ISP- 6), Modified Nutrient Glucose Agar (MNGA) and Nutrient Agar (NA), Potato Dextrose Agar (PDA), Starch Agar, Czapek Dox Agar (CZ Agar) and Modified ISP-2 (MISP-2) procured from Hi-Media, India after 1-2 weeks incubation at 28 ºC
(Williams, 1989; Dubey and Maheshwari, 2008; Goodfellow et al, 2012).
Colony characters were studied using Stereomicroscope (ZEISS-Axio cam MRe, Biodiscovery.V20, Germany). Morphological parameters were also studied by light microscope (Leica ATC, 2000) and scanning electron microscope (ZISS, Evo /LS15, Germany) (Williams, 1989 ; Goodfellow et al, 2012).

Biochemical and physiological parameters

Various biochemical and physiological tests viz. Catalase, Oxidase, utilization of Citrate, Nitrate reduction, Starch and Gelatin hydrolysis tests were performed on ACTM-8 (Dubey and Maheshwari, 2008). Production of melanin pigmentation on ISP-6 was performed on ACTM-8using standard media fromHi media, Mumbai, India (Shirling and Gottlieb,1966). Thin layer chromatography was performed to analysis of 2-6 diaminopimelic acid (DAP) present in the whole cell wall composition of the ACTM-8 (Hasegawa et al, 1983).

DNA isolation

The isolate of actinomycetes strain ACTM-8 were grown in ISP-2 broth medium for 94 hours at 28 ^{o}C. Mini Isolation Kit procured from Amnion Biosience in India used for isolation of genomic DNA.

Amplication and sequencing of 16S rRNA Gene

The Polymerase Chain Reaction (PCR) was used to amplified the 16S rRNA gene with the bacterial primer pairs 27F (5-AGAGTTTGATCCTGGCTCAG-3) and 1492R (5-GGTTACCTTGTTACGACTT-3) (Weisburg et al, 1991). The procedure was done in the Thermal Cycler (Applied Biosystems, U.S.A), in the volume of 50 µl containing 50ng/µl DNA, 100pmol each primer, 10mM dNTPs, 2 µl 10X PCR buffer and 0.25 Unit *Taq*DNA polymerase (Amnion PCR Research kit, India). PCR conditions include; primary denaturation at the temperature of 94 ^{o}C for 5 min followed by denaturation at 94 ^{o}C for 30 sec, then 55 ^{o}C for 30 sec as a primer annealing, 72 ^{o}C for 1.5 min as a primer extension and 72 ^{o}C for 5 min as the final extension in 35 cycles. The PCR products were visualized using Ultraviolet (UV) fluorescence gel documentation system (UTP-Bio Doc, USA) on 1.5 % (w/v) agarose gel along with 5 Kb DNA ladder as a marker. Sequencing of PCR product was carried out after PCR product cleanup. The eluted purified PCR products were, subsequently sequenced by an automated gene sequencer (3730xl DNA analyzer, Applied Biosystems, U.S.A).

Analysis of phylogenetic

The Blast program (www.ncbi.nlm.nih.gov/blst) used to assess the level of similarity of the strain ACTK-8 with other strains. The Bio Edit program used to evaluate Multiple sequence alignment and molecular phylogeny. The phylogenic tree was derived using neighbor-joining method on a bootstrap dataset containing 100 replicates (Hall, 2000).

In vitro antimicrobial bioassay

The antimicrobial activity of strain ACTM-8 was carried out in of two steps, primary screening and secondary screening.
The microorganisms used included Gram-positive bacteria *Staphylococcus aureus* (MTCC-96), *Bacillus subtilis* (MTCC 121) and Gram-negative bacteria *Escherichia coli* (MTCC 729), *Enterobacter aerogenes* (MTCC 2829). All the

bacterial cultures were obtained from the Microbial Type Culture Collection (MTCC), Chandigarh, India. The filamentous fungi used as cereal fungal pathogens employed include, *Fu*sarium *acminatum*(KJ371100), *F.sporotrichioides*(KJ371098),*F.equeseti*(KJ371094),*F.venenatum*(KJ371103),*F.poea*(KJ371096), *F.graminarium*(KJ371099),*F.avenacium*(KJ371102),*F.sambucinu*(KJ371095),*F.nivale* (KJ371097),*F.crookwellense*(KJ371105),*F.anthophilum*(KJ371093),*F.semitectum*(KJ37110),*F.arminacium*(KJ37110) and*F.culmorum*(KJ371104) were obtained by the DOS in Microbiology, Mysore university, ,Karnataka state ,India.

Preliminary screening

Antibacterial activity

Primary screening of antibacterial activity of strain ACTM-8 was determined by using perpendicular streak method (Egorov, 1985) against the test bacteria . The strain ACTM-8 was streaked as a straight line on the middle of ISP-2 medium and the plates were incubated at 28 ^{0}C. After 6 days incubation, the test bacteria were inoculated perpendicular to the strain ACTM-8 and the plates were incubated for 24h at 37^{o} C. The zone of inhibition was determined using a millimeter scale in millimeter (mm).

Antifungal activity

Dual culture method (Sinclair and Dhingra, 1995) was used for antifungal activity of strainACTM-8. The strain ACTM-8 was streaked as a straight line on the middle of MISP-2 plates and incubated at 28 ^{o}C.After 6 days incubation,plates were aseptically inoculated with fungal mycelia-discs (6 mm in diameter) prepared from growing margin of freshly cultured *Fusarium* spp. on PDA and incubated at 25±2 ºC for 5-7 days. Antifungal activity was assayed by inhibition of mycelia growth of tested fungi toward of active strain ACTM-8. The degree of inhibition at duel cultures was evaluated as follows: +++ (> 20 mm, strong inhibition), ++ (10-19 mm, moderate inhibition) and + (2-10 mm, weak inhibition). Controls included fungal mycelium plugs in center of non- *Streptomyces* inoculated MISP-2 plates.

Secondary screening

Based on the results of primary screening, the strain ACTM-8 showed broad antimicrobial activity against test organisms and the strain was subjected to secondary screening.

Fermentation conditions and extraction of antifungal compounds

The Erlenmeyer flask (250 ml) containing 50 ml of modified ISP-2 Broth medium was used for fermentation (Shuler and Kargi, 1992). 100 µl of the standardized actinomycetes suspension (1.5 ×10^{5} CFU/ µl) was used to inoculate the fermented broth and incubated in an orbital shaker (100 rpm) for 10 days at 28 ºC. After the incubation period, fermented broth was filtrated through Watchman No.1 filter paper. Then, the supernatant of the fermented broth of the strain ACTM-8 was separated and subjected for extraction of antifungal compound by liquid-liquid extraction method (Liu et al, 1986). Ethyl acetate (SD Fine chemicals limited, Mumbai, India)in the ratio of 1:1(v/v) was added to the supernatant of the strain ACTM-8 into separate funnel and shaken vigorously for 45 min to complete the extraction. The organic phase having antifungal property was separated from the aqueous phase. It was concentrated by subjecting to rotator flash evaporator at the 55 ºC at 200 rpm under vacuum. The residue (crude extract) obtained was dissolved in 1 ml of Dimethyl Sulfoxide (DMSO, E. Merck Ltd., Mumbai, India) and stored at 4 ºC for further analysis.

Antimicrobial activity assay

The disc paper diffusion method (Bauer et al, 1966) used for the antimicrobial activity of the ethyl acetate crude extract of strain ACTM-8.

20 ml of sterilized molten NA and PDA were aseptically poured into Petri dishes and allowed to solidify.100 µl from cell/spore suspension (1.5×10^{-6} CFU/ml) of test bacterial and fungal cultures were inoculated on the solidified NA and PDA media. A sterile filter paper disc (6mm, Himedia, Mumbai, India) was loaded aseptically with 50 µl of the crude extract of the strain ACTM-8 and placed over the surface of NA and PDA plates. All the plates incubated at 37± 2^{o}C and 25±2 ^{o}C for 24h and 72h for bacteria and fungi respectively. Streptomycin (10 µg/disc) and Nystatin (10µg/disc) were utilized as positive control for antibacterial and antifungal activity respectively and DMSO was utilized as negative control. After the incubation time, the inhibition zone around the each disc was indicative of antimicrobial activity measured in millimeters (mm) scale.

Statistical analysis

SPSS package version 16 was used to derive mean ± standard deviation for the antimicrobial activity of the different isolates against test bacteria and cereal fungal pathogens.

RESULTS

The aerobic actinomycete designated as strainACTM-8 (*Accession number*KJ024097) obtained from Madikeri grassland of soil samples in Kodagu district, Karnataka state in India exhibited a wide spectrum antifungal

activity against some cereal fungal pathogens. The strain ACTM-8 was Gram- positive filamentous bacterium (Figure 1). Culture properties of ACTM-8 carried out based on the observations conducted after 7, 14 and 21 days of incubation using different media. The aerial mycelia grew well to moderate on the tested media and produced powdery in velvety moss. The colors of filamentous aerial mycelium were bluish to whitish in color; it varied depending on the type of media used. The diffusible pigment on the media did not produced by the strain ACTK-8 only on ISP-2 medium which was brownish yellow (Table 1). Under both light and scanning electron microscope spiral chain of spores was observed (Figure 1). These cultural and morphological properties suggest that the strain ACTM-8 related to the genus *Streptomyces*.

Table-2 exhibits the physiological and biochemical properties of ACTM-8. The strain ACK-8 hydrolyzed starches, reduced nitrate, liquefied gelatin and utilized citrate but, it did not produce hydrogen sulphide (H_2S). It showed positive in Catalase and negative in Oxidase tests. production of melanin was seen on ISP-6 medium. The composition of cell wall of the strain ACTM-8 was obtained to be L-DAP (chemotype-I). All these characteristics are indicative of the genus *Streptomyces* (Table 2).

The nucleotide sequences of alignment (1489bp) of ACTM8.3 in the gene bank using NCBI revealed 99% similarity with *Streptomyces chartreuse* (*Accession number* KJ024097). The Figure 2 shows the phylogenetic tree by using the neighbor joining method.

The result of primary screening of antimicrobial activity of ACTM-8 showed wide spectrum antimicrobial activity against both tested pathogenic bacteria and fungi (Table 3). Out of 14 test *Fusarium* spp., the strain ACTM-8 showed antifungal activity against only 8 species of *Fusariumas*such as *F.graminarium, F.navile, F.poea*,*F.equeseti, F.sporotrichioiode, F. acminatum, F.culmorum*and*F.crookwellense* (Table 4).

In secondary screening for antibacterial activity, ethyl acetate extract of strain ACTM-8 showed maximum activity against *Ent.aerogenes* 12 .16± 0.28 mm followed by *B.subtilis* 10.66± 0.57mm and *Staph. aureus*10.33 ± 0.57 mm. However, the strain ACTM- 8 did not show any activity against *E. coli* (Table 3). For antifungal activity, ethyl acetate extract of strain ACTM-8 was most active against *F.graminarium* (36.33 ± 0.57mm) followed by *F.poea*(33.1±0.11 mm), *F.sporotrichioiodes*(30.26 ± 0.57 mm), *F.equeseti*(24.3 ± 0.17 mm) and *F.nivale* (18.33± 0.11 mm)respectively(Table 4).While, the strain ACTM-8 did not show any activity against *F.venenatum, F.avenacium, F.sambucinum, F.crookwellense, F.anthophilum,F. acminatum, F.arminacium, F.culmorum*and *F.semitectum*(Table 4).

DISCUSSION

The isolation of novel actinomycetes may help into the finding of newer, secure, impressible and a wide range of antimicrobial compounds as section of the strategy for inhibition of pathogenic infection (Demain and Sanchez, 2009; Livermore et al, 2011). In the period of screening of terrestrial *Streptomyces* spp. for antibiotic producing actinomycetes against pathogenic bacteria and cereal fungal pathogens, a wide spectrum of antimicrobial producing actinomycetes, the *Streptomyces* sp. strain ACTK-8 was isolated from Madikeri grassland of soil samples in the Kodagu district of Karnataka (India). The isolate ACTK-8 was identified through a combination of phenotypic and molecular methods. 16S rRNA gene sequence (1489bp) analysis of the strain ACTM-8 revealed 99% similarity with the 16S rRNA gene of *S.chartreusis* (KJ024097). In this study, *S. chartreusis* was assessed for antimicrobial activity agaist 4 Gram-positive and Gram-negative bacteria and 14 species of pathogenic *Fusarium*. Primary screening of strain ACTM-8 showed wide spectrum antimicrobial activity against both test bacteria and fungi.Hence, the isolate was subjected to secondary screening using liquid-liquid extraction method. ACTM-8 secreted antimicrobial compounds in liquid culture which was extracted using ethyl acetate (1:1, v/v) from the supernatant of 10 days old culture broth of *S. charteousis* strain ACTM- 8.

The effectiveness of ethyl acetate extract of strain ACTM- 8 was found to be varied against in different test bacterial (both Gram-positive and Gram-negative bacteria) and fungal organisms. Further, ethyl acetate extract of strain ACTM-8 exhibited activity against 5 species of *Fusarium* such as *F.graminarium* followed by *F.navile, F.poea*,*F.equeseti*and*F.sporotrichioiodes*which are a major phytopathogens leading to greater economic crisis due deterioration of nutritive value of cereals (Munkvold and Desjardins, 1997). Among the organisms tested, *F.graminarium,* a major cause of *Fusarium* Head Blight (FHB) was significantly inhibited with antifungal compound produced by strain ACTM-8.

Similar antagonistic approaches have also been reported in different species of *Streptomyces* from different habitats (Prapagdee et al, 2008; Atta and Reyad, 2013; Kanini et al, 2013). The study findings revealed that there is a difference in antimicrobial activity of the strain ACTM-8 in

both primary and secondary screening. It could be because of differences
in the type of growth of actinomycetes in agar media as filamentous form and broth media as a fragmenting form and also other chemical modification of the bioactive compounds to become inactive in broth culture (Pickup et al, 1993). The results confirmed that the composition of the medium could be affected on the antimicrobial compounds. Similar findings have been reported by a few investigators (Usha et al, 2011; Dezfully and Ramanayaka , 2015).
Our results strongly support the idea that species of *Streptomyces* possess a significant ability to produce bioactive compounds having, unique therapeutic applications (Watve, 2001; Lam, 2007).

CONCLUSION

This study is an effort to characterize the potent antimicrobial strain of actinomycetes isolated from the unexplored soil of Kodagu district and also evaluate its antimicrobial activity against test pathogenic organisms. Findings revealed that *S. chartreusis* strain ACTM-8 as the potent wide spectrum antimicrobial compound isolated from unexplored soil of Kodagu district could be as a promising candidate for developing into a potent antibiotic. However, further investigation on purification and structural characterization of the antimicrobial active compounds will be useful for pharmaceutical and agricultural applications.

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FINANCIAL DISCLOSURE

Non

FOOTNOTES

Isp: International *Streptomyces* Project.

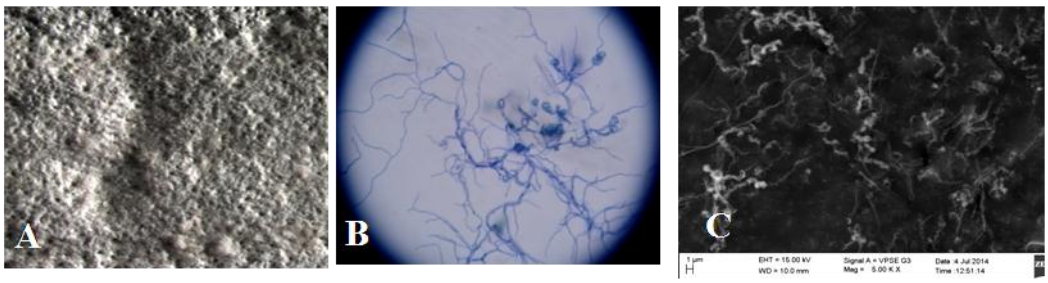

Fig. 1: (A) Stereomicroscopic, (B) Micromorphologic (under 100X) and (C) Scanning Electron Micrographs of *S.chartreusis* strain ACTM-8 on ISP-2 agar medium (bar:1µm).

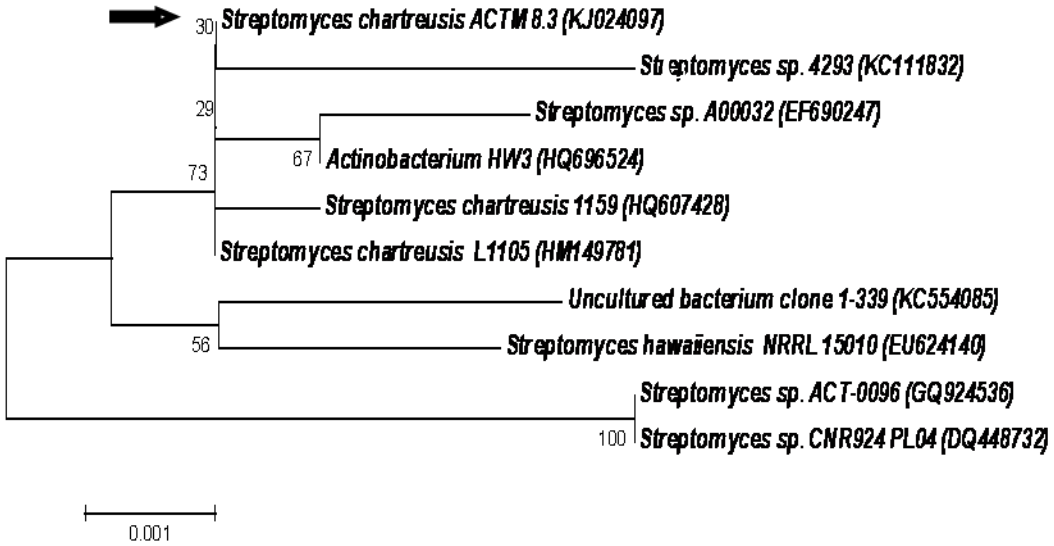

Fig. 2: 16S rRNA showing the phylogenetic relationship by neighbor-joining method between strain ACTM- 8 (KJ024097) with other known *Streptomyces spp.*

Table 1: Culture characteristics of *Streptomyces chartreusis*isolate ACTM-8 in different media after 14 days of incubation at 28ºC

Sl. No.	Medium	Growth	Substrate mycelium	Aerial mycelium	Spores	Soluble pigment
1	**ISP-1***	Abundant	Yellowish brown	bluish	Blue	-
2	**ISP-2**	Abundant	Yellowish brown	Medium bluish	Blue	Brownish yellow
3	**ISP-3**	Abundant	Yellowish white	Bluish	Whitish blue	-
4	**ISP-4**	Abundant	Whitish	Light bluish	Bluish white	-
5	**ISP-5**	Moderate	Yellowish white	Yellowish white	Whitish	-
6	**ISP-6***	Abundant	Dark brown	Dark brown to black	Dark brown to black	Dark brown to Black
7	**MNGA**	Medium	Yellowish white	-	-	-
8	**NA**	Medium	Yellowish white	-	-	-
9	**PDA**	Abundant	Brownish	Bluish	Bluish	-
10	**Starch Agar**	Abundant	Yellowish white	Whitish	Whitish	-
11	**CZ Agar**	Moderate	Yellowish white	Whitish	Whitish	-
12	**MISP-2**	Abundant	Yellowish white	Bluish	Bluish	-

Note: *ISP-1-6: International Streptomyces Project; MNGA: Modified Nutrient Glucose Agar; MISP-2: Modified ISP-2 Agar; PDA: Potato Dextrose Agar; CZ Agar: Czapek Dox Agar.

Table 2: Biochemical and Chemotaxonomic characteristics of *S. chartreusis* ACTM-8 isolated from soil samples of Kodagu district, Karnataka (India)

Sl. No.	Properties of *Streptomyces chartreusis strain* ACTM-8	
I.	**Biochemical characteristics**	
1	Catalase test	**+**
2	Oxidase test	**-**
3	Citrate utilization	**+**
4	Gelatin hydrolysis	**+**
5	Nitrate reduction	**+**
6	Starch hydrolysis	**+**
7	Melanin production	**+**
II.	**Chemotaxonomic characteristics**	
1	Cell wall amino acid analysis	L-DAP*
III.	Grams staining	**+**

Note: *L-DAP: L- *diaminopimelic acid*

Table 3: Antibacterial and Antifungal activity of strain ACTM-8 against test bacteria

SL.NO	Bacterial pathogens	Antibacterial activity		
		Primary screening	Secondary screening (Zone of inhibition in mm)	
			Ethyl acetate extract (100 µg/ml)	Streptomycin (10µg/disc) (positive control)
1	*Staph.aureus*(MTCC- 96)	6	10.33 ± 0.57	24.16±0.28
2	*B. subtilis*(MTCC 121)	7	10.66± 0.57	29.16 ±0.18
3	*E. coli* (MTCC 729)	-	-	26.33±0.57
4	*Ent. aerogenes*(MTCC2829)	9	12 .16± 0.28	28.5 ±0.5

Note: Results in the secondary screening are mean ± Standard Deviation (SD) of the three replicate experiments ; "-": No activity.

Table 4: Antifungal activity of strain ACTM-8 against test fungi (*Fusarium* spp.)

SL.NO	Fungal pathogens	Antifungal activity		
		Primary screening	Secondary screening (Zone of inhibition in mm)	
			Ethyl acetate extract (100mg/ml)	Nystatin (10µg/disc) (positive control)
1	*F. acminatum* (KJ371100)	+	-	-
2	*F.sporotrichioides* (KJ371098)	++	30.26 ±0.57	20.6 ±0.17
3	*F.equeseti* (KJ371094)	++	24.3± 0.17	15.6 ±0.11
4	*F.venenatum* (KJ371103)	-	-	-
5	*F.poea* (KJ371096)	++	33.1 ±0.11	12.83 ±0.11
6	*F.graminarium* (KJ371099)	++	36.33 ± 0.57	22.5 ±0.5
7	*F.avenacium* (KJ371102)	--	-	-
8	*F.sambucinum* (KJ371095)	--	-	-
9	*F.nivale* (KJ371097)	++	18.33 ± 0.11	–
10	*F.crookwellense* (KJ371105)	+	-	-
11	*F.anthophilum* (KJ371093)	-	-	-
12	*F.semitectum* (KJ371106)	-	-	-
13	*F.arminacium* (KJ371101)	-	-	-
14	*F.culmorum* (KJ371104)	+	-	-

Note: Results in the secondary screening are mean ± Standard Deviation (SD) of the three replicate experiments ; "-": No activity.

REFERENCES

1. Aravind N A, Tambat B, Ravikanth G, Ganeshaiah KN and Shaanker RU. Patterns of species discovery in the Western Ghats, a megadiversity hot spot in India. J Biosci. 2007;32(4):781-790.
2. Atta HM and Reyad AM. Antifungal agent from Streptomyces sp: Taxonomy, Fermentation, Purification and Biological Activities. Nature Sci. 2013;11(11):164-176.
3. Bauer AW, Kirby WMM, Sherris JC and Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966; 45(4): 493.
4. Berdy J. Bioactive microbial metabolites. J Antibiot.2005;58(1): 1.
5. Demain AL and Sanchez S. Microbial drug discovery: 80 years of progress. The J Antibiot. 2009; 62(1): 5-16
6. Dezfully NK and Heidari A. Natural bioactive compounds: Antibiotics. J Fundam Appl Sci. 2016;8(2S):674-684.
7. Dezfully NK and Ramanayaka JG. Isolation, identification and evaluation of antimicrobial activity of Streptomyces flavogriseus, strain ACTK2 from Soil Sample of Kodagu, Karnataka State (India). Jundishapur J Microbiol. 2015; 8(2).
8. Dubey RC and Maheshwari DK. . Practical Microbiology. New Delhi; S. Chand and Company Ltd. 2008; 2nd ed.
9. Egorov NS. Antibiotics: A scientific approach. MIR publishers. 1985.
10. El-Nakeeb MA and Lechevalier HA. Selective isolation of aerobic actinomycetes. Appl Microbiol. 1963;11(2):75-77.
11. Goodfellow M,Whitman WB, Parte AC and Kampfer P. The Actinobacteria. In: Bergey's Manual of Systematic Bacteriology, Vol.5, New York: Springer. 2012; 2nd ed.
12. Hall T. BioEdit: Biological sequence alignment editor for windows 95/98/NT, 4.8.2000.
13. Hasegawa T, Takizawa M and Tanida S. A rapid analysis for chemical grouping of aerobic actinomycetes. J Gen Appl Microbiol. 1983;29(4):319-322.
14. Kanini GS, Katsifas EA, Savvides AL and Karagouni AD. Streptomyces rochei ACTA1551, an indigenous Greek isolate studied as a potential biocontrol agent against Fusarium oxysporum f. sp. lycopersici.Biomed Res Int. 2013;2013 :1-10.
15. Khandan ND and Janardhana GR. Diversity and antimicrobial activities of actinomycetes isolated from soil samples of Kodagu, Karnataka state (India). Int J Microbiol Res. 2013;5(3):404.
16. Lam and Kin S. New aspects of natural products in drug discovery.Trends Microbiol. 2007;15:279-289.
17. Liu CM, Westley JW, Hermann TE, Prosser BLT, Palleroni N, Evans RH and

Miller PA. Novel polyether antibiotics X-14873A, G and H produced by a Streptomyces: Taxonomy of the producing culture, fermentation, biological and ionophorous properties of the antibiotics. J Antibiot.1986: 1986;39(12):1712-1718.
18. livermore DM ,Blaser M, Carrs O, Cassell G, Fishman N, Guidos R, Levy S,Powers J, Norrby R,Tilloston G, Davis R, Projan S, Dawson M, Monnet D, Keogh-Brown M, Hand K, Garner S, Findly D and White T. Discovery research: the scientific challenge of finding new antibiotics. J Antimicrob Chemother. 2011;66:1941-1944.
19. Maciejewska M , Pessi IS, Arguelles-Arias A, Noirfalise P, Luis G, Ongena M, Barton H, Carol M and Rigali S. Streptomyces lunaelactis sp. nov., a novel ferroverdin A-producing Streptomyces species isolated from a moonmilk speleothem. Anton Leeuw. 2015;107(2):519-531.
20. Munkvold GP and Desjardins AE. Fumonisins in maize: can we reduce their occurrence. Plant Disease. 1997;81(6):556-565.
21. Pickup KM, Nolan RD and Bushell ME. A method for increasing the success rate of duplicating antibiotic activity in agar and liquid cultures of Streptomyces isolates in new antibiotic screens. J Ferment Bioeng. 1993;76(2):89-93.
22. Prapagdee B, Kuekulvong C and Mongkolsuk, S. Antifungal potential of extracellular metabolites produced by Streptomyces hygroscopicus against phytopathogenic fungi. International Journal of Biological Sciences. 2001 ;4(5):330-337.
23. Rosselló-Mora R and Amann R. The species concept for prokaryotes. FEMS Microbiol Rev. 2001;25(1):39-67.
24. Saintpierre-Bonaccio D, Maldonado LA., Amir H, Pineau R and Goodfellow M. Nocardia neocaledoniensis sp. nov., a novel actinomycete isolated from a New-Caledonian brown hypermagnesian ultramafic soil. Int J Syst Evol Microbiol. 2004;54(2):599-603.
25. Shirling ET and Gottlieb D. Methods for characterization of Streptomyces species1. Int Jof Syst Evol Microbiol. 1996;16(3):313-340.
26. Shuler ML and Kargi F. Bioprocess engineering, Basic Concept. New Jersey: Prentice-Hall. 1992; 1st ed.
27. Sinclair JB and Dhingra OD. Basic Plant Pathology Methods. CRC press. 1995.
28. Tiwari K. and GuptaRK. Rare actinomycetes: a potential storehouse for novel antibiotics. Crit Rev Biotechnol. 2012;32(2):108-132.
29. Tsao PH, Leben C and Keitt G. An enrichment method for isolating Actinomycetes that produce diffusible antifungal antibiotics.Phytopathol. 1960;50(1):88-89.
30. Usha Kiranmayi M, Sudhakar P, Sreenivasulu K. and Vijayalakshmi M. Optimization of culturing conditions for improved production of bioactive metabolites by Pseudonocardia sp. VUK-10. Mycobiol. 2011;39(3):174-181.
31. Veyisoglu A, Tatar D, Cetin D, Guven K and Sahin N. Streptomyces karpasiensis sp. nov., isolated from soil. Int J of Syst and Evol Microbiol. 2014;64(3):827-832.
32. Watve MG, Tickoo R, Jog MM and Bhole BD. How many antibiotics are produced by the genus Streptomyces?. Arch Microbiol. 2001;176(5):386-390.
33. Weisburg WG, Barns, SM, Pelletier DA and Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol. 1991;173(2):697-703.
34. Williams ST. Genus Streptomyces waksman and henrici 1943.Bergey's Manual of Syntematic Bacteriology. 1989;4:2452-2492.