Nithya K. *et al.* / *International Journal of Biological & Pharmaceutical Research.* 2016; 7(2): 116-121.

e- ISSN 0976 - 3651
Print ISSN 2229 - 7480

International Journal of Biological & Pharmaceutical Research
Journal homepage: www.ijbpr.com

IJBPR

ISOLATION, CHARACTERIZATION AND SCREENING OF ANTIMICROBIAL METABOLITES FROM DESERT ACTINOBACTERIA *STREPTOMYCES* SP. STRAIN DA7-2

Nithya K[1], Muthukumar C[1], Dhanasekaran D[1*], ArunachalamC[2] and Thajuddin N[1]

[1] Department of Microbiology, School of Life Sciences, Bharathidasan University, Tiruchirappalli 620024, Tamil Nadu, India.
[2] Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia.

ABSTRACT

An antimicrobial bioactive metabolites producing actinobacterial strain DA7-2 was isolated from desert soil samples. Based on morphological and biochemical characterization, the isolate was identified as *Streptomyces* sp. strain DA7-2. Based on the media selection, modified nutrient glucose medium was selected for the production of bioactive metabolites. Ethyl acetate extract of DA7-2 exhibited good antimicrobial activities against pathogenic bacteria such as *K. pneumoniae* (15 mm), *E. faecalis* (11 mm), *E. coli* (13 mm), *S. typhimurium* (12 mm), *P. aeruginosa* (8 mm), *S. aureus* (18 mm) and fungi such asC. *albicans* (8 mm)*C. neoformans* (12 mm) and *S. cerevisiae* (17 mm). Based on the results of antimicrobial activity against pathogenic microorganisms, it is clear that the actinomycetes from desert soil environs are promising source for discovery of new antimicrobial compounds.

Key Words: Desert soil, Actinobacteria, *Streptomyces*, Antimicrobial.

INTRODUCTION

Due to the need of novel antibacterial agents, the desert actinobacteria are often searched and screened for the production of highly effective antibacterial agent with novel structure. Among actinobacteria, the genus *Streptomyces* alone produced around7, 600 bioactive compounds. They can produce 80 % of total antibiotics currently available in the market and other secondary metabolites. The genus *Streptomyces* are majorgroups of industrially important organisms and have the ability to synthesize varieties of valuable products of commercial importance (Saha *et al*., 2012; Saha *et al*., 2013; Valli *et al*.,2012; Dhanasekaran *et al*., 2014; Nithya *et al.,* 2015).

Corresponding Author

Dhanasekaran D
Email: dhansdd@gmail.com

The antibiotic resistance and decrease in the rate of The antibiotic resistance and decrease in the rate of discovery of new antimicrobial compounds draws the attention of scientists to try to investigate unexplored habitats for novel actinomycetesas possible candidates of new antimicrobials. The recent discovery of novel primary and secondary metabolites from taxonomically unique populations of extremophilicactinomycetes suggest that, these organisms could add anew dimension to microbial natural product research (Thumar *et al*., 2010). Only fewer study in actinomycetes research in desert soil samples of Saudi Arabia (Atta *et al*., 2010; Al-Habib and Magda, 2012; Nithya *et al*., 2015). Therefore, in recent years desert actinomycetes have attracted the attention of interested scientists. Owing to their diversity in biological activities and production of novel chemical compounds such as antibiotics like other microorganisms that inhabit extreme environments actinomycetes are promising (Kumar and Kannabiran, 2010; Vijayakumar *et al.,* 2012;

Dhanasekaran *et al.*, 2014).The present investigation was aimed to isolation actinobacteria from desert soil samples and screening for its antimicrobial activity.

MATERIALS AND METHODS

Sample collection and isolation of actinobacteria

The desert soil samples totally ten different places were collected from Riyadh, Saudi Arabia using sterile polythene bags and transported to the laboratory for further analysis. Actinobacteria was isolated using starch casein agar medium along with antibiotics such as Nalidixic acid (10 µg/ml) and Amphotericin B (20 µg/ml) to prevent the bacterial and fungal growth respectively. The collected soil samples were serially diluted and 0.5 ml of dilutions (10^{-3} and 10^{-4}) was inoculated over the plate. The plates were incubated at 28^{o}Cfor 7–15 days. The colonies of actinobacteria developing onthe SCA plates were purified and maintained in SCA slants.

Pathogens

Totally seven pathogenic bacteria and three pathogenic fungi were obtained from American type culture collection (ATCC, USA). The name of the pathogens and their ATCC number are listed in Table 2.

Screening of antimicrobial activity of actinobacteria

The preliminary screening of antimicrobial activity was carried out by cross-streak plate method (Egorov, 1985) against pathogenic bacteria and yeast like fungi. A single streak was made at the centre of plates containing ISP-2 medium and incubated at 28 ^{o}C for 8 days and then the pathogenic bacteria and fungi were streaked at right angles to the original streak of actinobacteria and the plates were again incubated at 37 ^{o}C for 24 to 48 h. Based on the results of inhibition zone, the isolates DA 7-2 was selected for further studies.

Cultural characterization of the isolate DA 7-2 Morphological and Biochemical

The morphological characteristics of the strain DA3-7 was studied in the following culture media as recommended by the International *Streptomyces* Project (ISP) including ISP 1 to 7, starch casein agar, nutrient agar and glucose yeast extract agar (Shirling and Gottlieb, 1966). The growth, colour of the aerial and substrate mycelium, reverse side colour and diffusible pigment production were observed and documented.

Biochemical characterization of the isolate DA7-2

Biochemical characteristics such as indole, methyl red, Vogesproskauer, citrate utilization, catalase, oxidase, triple sugar iron, urease, casein, gelatin, lipid and starch hydrolysis were carried out.

Media selection for bioactive compounds production

The selected potential isolate DA 7-2 was screened their bioactive compounds production in five different media namely ISP 2 (yeast extract malt extract), M6 (fermentation medium), MNG (modified nutrient glucose), SD (sabouraud dextrose) and YPG (yeast peptone glucose) media. The isolate DA 7-2 was inoculated the above media and incubated at 28 ^{o}C in rotary shaker at 120 rpm for 8 days. The fermented broth was tested against pathogenic bacteria and fungi by agar well diffusion method (Thirumurugan and Vijayakumar, 2013).

Fermentation and extraction of bioactive metabolites

From the media selection, the isolate DA 7-2 showed good antimicrobial activity and produced bioactive metabolites in MNG medium and therefore, the MNG medium used for fermentation studies. The isolate DA 7-2 (5%) was inoculated in 700 ml of MNG media and incubated at 28 ^{o}C in a rotary shaker at 120 rpm for 8 days. The fermented broth was centrifuged in cooling centrifuge (4 ^{o}C) at 5,000 rpm for 10 min, filtered through What-man No. 1 filter paper and then supernatant was collected. The equal volume of ethyl acetate (v/v) was added along with supernatant and shaken vigorously. Allowed 10 min for settle of layers, the upper organic layer which contained the bioactive compounds was separated by using separating funnel. The supernatant kept in rotary evaporatorat 60 ^{o}C to get a concentrated viscous extract. The crude extract was collected and stored at 4^{o}C for further analysis.

Antimicrobial assay (Disc diffusion method)

Ethyl acetate extract of selected actinobacterial isolate DA 7-2 was subjected to antimicrobial efficacy against pathogenic bacteria and fungi by disc diffusion method (Bauer et al., 1966). The suspensions of pathogenic bacterial and fungi were adjusted to 106 CFU/ml and swapped over Muller-Hinton agar. The DA7-2 ethyl acetate extract was loaded to the sterile disc with concentration of 5 mg/disc in DMSO solvent and placed over to the pathogenic bacterial and fungi inoculated plates. After 24–48 h of incubation at 37 ^{o}C and 28^{o}C for bacteria and fungi respectively, the diameter of the zone of inhibition was measured to evaluate the antibacterial assay of theDA7-2 extract. The experiments were performed with three replicates, and the mean values along with the standard deviation of the concerned data (n = 3) are presented in the results. Streptomycin (10 µg/disc) and Amphotericin B (25 µg/disc) were used as positive control for bacterial and fungi respectively and DMSO was used as solvent for negative control.

Table 1. Morphological and biochemical characterization of *Streptomyces* sp. strain DA7-2

Characteristics	Results
Gram's stain	Positive
Shape and growth	Filamentous aerial growth
Aerial mycelium	Whitish ash
Substrate mycelium	Yellowish brown
Diffusible pigment	-
Growth temperature	25 to 40 ^{o}C
Indole	-
Methyl red	-
Vogesproskauer	-
Citrate utilization	-
Triple sugar iron agar	-
Nitrate	-
Urease	+
Catalase	+
Oxidase	-
Starch hydrolysis	+
Gelatin hydrolysis	-
Lipid hydrolysis	-
Casein hydrolysis	-

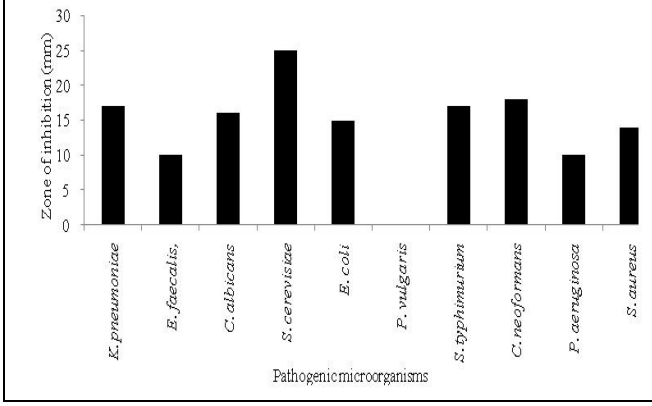
+, positive; -, negative

Table 2. Antimicrobial activity of *Streptomyces* sp. strain DA7-2 ethyl acetate extract against pathogenic microbes

S.No.	Test organisms	Strain number	Zone of inhibition (mm)	
			Crude extract DA7-2 (5mg/disc)	Control
	Bacteria			Streptomycin (10 µg/disc)
1.	*K. pneumoniae*	ATCC 12882	15	17
2.	*E. faecalis*	ATCC 49532	11	15
3.	*E. coli*	ATCC 10536	13	16
4.	*P. vulgaris*	ATCC 33420	-	17
5.	*S. typhimurium*	ATCC 13311	12	14
6.	*P. aeruginosa*	ATCC 27883	8	20
7.	*S. aureus*	ATCC 6538P	18	21
	Fungi			Amphotericin B (25 µg/disc)
8.	*C. albicans*	ATCC 2091	8	10
9.	*C. neoformans*	ATCC 90113	12	14
10.	*S. cerevisiae*	ATCC 9763	17	-

- no activity.

Fig 1. Antimicrobial activities of *Streptomyces* sp. strain DA7-2: a) preliminary screening; b) cross streak method. 1 - *K. pneumoniae,* 2 -*E. faecalis,* 3 -*C. albicans,*4 - *S. cerevisiae,*5 - *E. coli,*6 - *P. vulgaris,* 7 -*S. typhimurium,*8 - *C. neoformans,* 9 -*P. aeruginosa,*10 - *S. aureus*



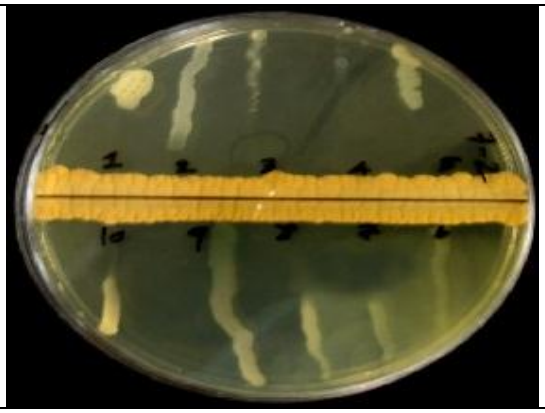
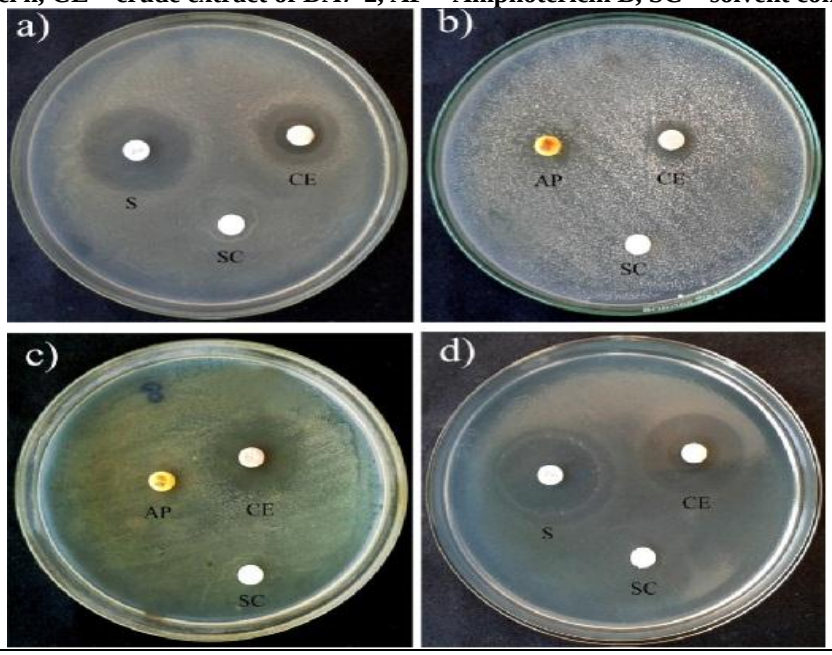


Fig 2. Antimicrobial activities of crudeextract of DA7-2: a) *K. pneumonia,* b) *C. albicans,*c) *C. neoformans,* d) *S. aureus.* S – Streptomyci n, CE – crude extract of DA7-2, AP – Amphotericin B, SC – solvent control DMSO.



RESULTS AND DISCUSSIONS

Isolation and primary screening of actinobacteria

Actinobacteria are one of the most vibrant bioactive compound producers among the microbial community. Desert soils are one of the suitable environments for isolation of many novel actinobacteria which could be good source for potentially useful active metabolites and biotechnological applications (Hozzein *et al*., 2008).Altogether 134 actinobacterial isolates, only 16 isolates were exhibited the antimicrobial potential based on preliminary screening (cross streak method). Among them, DA7-2 showed broad spectrum antimicrobial activity including both pathogenic bacteria and also yeast like fungi (Fig. 1).Previously many researchers have been reported that *Streptomyces* produced antimicrobial metabolites (Boudemagh *et al.,* 2005; Fguira *et al.,* 2005; Saravana Kumar *et al.,* 2014; Govindarajan *et al.,* 2014). Similarly, actinomycetes from unexplored or under explored environs including marine, desert and forest ecosystem are screened their antimicrobial potential (Berdy, 2005; Atta *et al.,* 2010; Radhakrishnan *et al.,* 2010).

Morphological and biochemical characterization of the isolate DA7-2

The strain DA7-2 was filamentous gram-positive actinobacteria and morphology in ISP-2 medium formed whitish ash in colour aerial mycelium and reverse side showed yellowish brown in colour. The strain DA7-2 not produced diffusible pigments in SCA and ISP-2 medium. The isolate was able to grow well in 25 to 40 ^{o}C. Biochemical characteristics of the isolate DA7-2 showed only positive results in urease, catalase and starch hydrolysis whereas, the remaining test including indole, methyl red, Vogesproskauer, citrate utilization, oxidase, triple sugar iron, casein, gelatin and lipid showed negative results. The results of morphological and biochemical properties revealed that the isolate DA7-2 belonged to *Streptomyces* sp. The obtained results (Table 1) were compared to the relevant characteristics in Bergey's Manual of Systematic Bacteriology(Whitman *et al.* 2012) for identifyi ng the strain DA7-2.

In vitro antimicrobial assay

The selected isolate DA7-2 was performed for screening of different media. Among them five media tested, MNG media was found suitable for antimicrobial compounds production and showed good antimicrobial activity. The crude extract of DA7-2 was prepared using MNG fermentation media and used for antimicrobial efficacy. The ethyl acetate extract of *Streptomyces* sp. DA7-2 was tested against pathogenic bacteria and fungi by disc diffusion method. Results revealed that good antimicrobial activity was observed in the ethyl acetate extract of strain DA7-2(Fig. 2). The extract inhibited the growth of pathogenic bacteria and fungi at a concentration of 5 mg/ disc for *K. pneumoniae* (15 mm), *E. faecalis* (11 mm), *E. coli* (13 mm), *S. typhimurium* (12 mm), *P. aeruginosa* (8 mm), *S. aureus* (18 mm), *C. albicans* (8 mm) *C. neoformans* (12 mm) and *S. cerevisiae*(17 mm). The ethyl acetate crude extract of DA7-2 was not inhibited the growth of *P. vulgaris* (Table 2). Many researchers have been reported that the secondary metabolites from actinobacteria are extracellular in nature (Saravana Kumar

et al. 2014; Dhanasekaran *et al.,* 2014). In this study, also confirmed that *Streptomyces* sp. strain DA7-2 produced bioactive metabolites are in extracellular product and also exhibited good activities against pathogenic bacteria and fungi. The solvent ethyl acetate has been used for extraction of extracellular products. Likewise, several studies have been proved ethyl acetate is suitable solvent for extraction of antimicrobial compounds from actinobacteria (Vijayakumar *et al.,* 2012; Saravana Kumar *et al.,* 2014). To find out the active chemical compounds, further studies need for separation, purification and characterization of antimicrobial compounds.

ACKNOWLEDGEMENT

The first author KN thankful to the UGC-RGNF for the award of SRF (UGC approval no: F1-17.1/2011-12/RGNF-SC-TAM-1376).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

Al-Habib RS, Magda AA.Physiological characteristics, sensitivity of antibiotics and inhibitors of soil alkalophilicactinomycetes of Al-Qassim Region Kingdom Saudi Arabia. *Journal of Agriculture and Veterinary Science*. 2013; 6: 43-55.

AttaHM, Bayoumi R, El-Sehrawi M, Aboshady A,Al-Humiany A. Biotechnological application for producing some antimicrobial agents by actinomycetes isolates from Al-khurmah Governorate. *European Journal of Applied Sciences*. 2010; 2: 98-107.

Bauer AW, Kirby WMM, Sherris JC, Truck M. Antibiotic suspectibility testing by a standardized single disk method. *American Journal of Clinical Pathology*.1966; 45: 493–496.

Berdy J. Bioactive microbial metabolites.*Journal of Antibiotics.* 2005; 58: 1–26.

Boudemagh A, Kitouni M, Boughachiche F, Hamdiken H, Oulmi L, Reghioua S, Zerizer H, Couble A, Mouniee D, Boulahrouf A,Boiron P. Isolation and molecular identification of actinomycetemicroflora, of some Saharian soils of south east Algeria(Biskra, EL-Oued and Ourgla)study of antifungal activity of isolated strains. J*ournal of Medical Mycology*. 2005; 15: 39–44.

Dhanasekaran D, Thajuddin N, Panneerselvam A,Chandraleka S. Isolation, characterization of antibacterial methyl substituted β-Lactum compound from *Streptomyces noursei* DPTD21 in Saltpan Soil, India. *Journal of Biologically Active Products from Nature*. 2014; 4: 71-88.

Egorov, NS. Antibiotics, a scientific approach.Mir Publishers, Moscow.1985.

Fguira LFB, Fotso Serge, Ameur-Mehdi R, Mellouli L, Laatsch H. Purification and structure elucidation of antifungal and antibacterial activities of newly isolated Streptomyces sp. strainUS80. *Research in Microbiology*. 2005; 156: 341–347.

Govindarajan G, Satheeja S, Jebakumar VSRD.Antimicrobial potential of phylogenetically unique actinomycete, *Streptomyces* sp. JRG-04 from marine origin. *Biologicals*. 2014; 42: 305–311.

Hozzein WN, Ali MIA, Rabie W.A new preferential medium forenumeration and isolation of desert actinomycetes. *World Journal of Microbiology and Biotechnology*. 2008; 24: 1547-1552.

Kumar S, Kannabiran K. Antifungal activity of *Streptomyces* VITSVK5 spp. against drug resistant *Aspergillus* clinical isolates from pulmonary tuberculosis patients. *Journal of Medical Mycology*. 2010; 20: 101–107.

Nithya K, Muthukumar C, DuraipandiyanV, Dhanasekaran D,Thajuddin N. Diversity and antimicrobial potential of culturable actinobacteria from desert soils of Saudi Arabia. *Journal of Pharmaceutical Science and Research*. 2015; 7: 117-122.

Radhakrshana M, Suganya S, Balagurunathan R, Vanaja Kumar. Preliminary screening for antibacterial and antimycobacterial. *World Journal of Microbiology and Biotechnology*.2010; 26: 561–566.

Saha S, Dhanasekaran D, ShanmugapriyaS, Latha S. *Nocardiopsis* sp. SD5: A potent feather degrading rare actinobacterium isolated from feather waste in Tamil Nadu, India. *Journal of Basic Microbiology*. 2013; 53: 608–616.

Saha S, Priyadharshini A, Dhanasekaran D, Thajuddin N, Chandraleka S, Chandramohan G, Panneerselvam A. Preclinical evaluation and molecular docking of 4-phenyl-1-Napthylphenylacetamide (4P1NPA) from *Streptomyces* sp. DPTB16 as a potent antifungal compound. *Computer in Biology and Medicine*. 2012; 42: 542-547.

Saravana Kumar P, Al-Dhabi NA, Duraipandiyan V, Balachandran C, Kumar PP, Ignacimuthu S. *In vitro* antimicrobial, antioxidant and cytotoxic properties of *Streptomyces lavendulae* strain SCA5. *BMC Microbiology*. 2014; 14: 291.

Shirling JL, Gottlieb D. Methods for characterization of *Streptomyces* species. *International Journal of Systematic and Evolutionary Microbiology.*1966; 16: 313–340.

Thirumurugan D, Vijayakumar R. A potent fish pathogenic bacterial killer *Streptomyces* sp. isolated from the soils of east coast region, South India. *Journal of Coastal Life Medicine*. 2013; 1(3): 175–180.

Thumar JT, Dhulia K, Singh SP.Isolation and partial purification of and antimicrobial agent from halotolerant alkaliphilic *Streptomyces aburaviensis* strain Kut-8. *World Journal of Microbiology and Biotechnology*. 2010; 26: 208.

Valli S, Sugasini S, Aysha OS, Nirmala P, Vinoth Kumar P,Reena A. Antimicrobial potential of actinomycetes species isolated from marine environment. *Asian Pacific Journal of Tropical Biomedicine*. 2012; 2(6): 469–473.

Vijayakumar R, Panneerselvam K, Muthukumar C, Thajuddin N, Panneerselvam A, Saravanamuthu R. Antimicrobial potentiality of a halophilic strain of *Streptomyces* sp. VPTSA18 isolated from the saltpan environment of Vedaranyam, India. *Annals of Microbiology*. 2012; 62: 1039-104.

Whitman W, Goodfellow M, Kämpfer P, Busse HJ, T ujillo M, Ludwig W, Suzuki K, Parte A (eds).Bergey's manual of systematic bacteriology, the actinobacteria. *Springer*. 2012; 1638–1763.