Journal of Pharmaceutical, Chemical and Biological Sciences
ISSN: 2348-7658
CODEN: JPCBBG
September - November 2018; 6(3):178-187
Online available at https://www.jpcbs.info

Research Article

OPEN ACCESS
The work is licensed under

Antimicrobial Activity Analysis of Bioactive Compounds from Soil Actinomycetes

Monisha Khanna Kapur[1]*, Renu Solanki[2], Payal Das[1], Munendra Kumar[1], Prateek Kumar[1]

[1]Acharya Narendra Dev College, University of Delhi, Govindpuri, Kalkaji, New Delhi 110 019, India
[2]Deen Dayal Upadhyaya College, University of Delhi, Sector 3, Dwarka, Opp NSIT, New Delhi 110078, India

***CORRESPONDING AUTHOR**
Dr. Monisha Khanna Kapur,
Principal Investigator, Microbial Technology Lab, Acharya Narendra Dev College University of Delhi, Govindpuri, Kalkaji, New Delhi 110 019, India
Email: monishaandc@gmail.com

ARTICLE INFORMATION
Received July 07, 2018
Revised August 29, 2018
Accepted September 04, 2018
Published October 03, 2018

ABSTRACT
Pathogenic microbes are becoming resistant to antibiotics used for their treatment. There is therefore an urgency to discover and develop new antimicrobial compounds with altered modes of action. Natural products from microorganisms are good source of new drugs. Among microbes, actinomycete bacteria are major producers of existing antibiotics. In the present study, actinomycetes were isolated from diverse ecological habitats and subjected to antimicrobial analyses. During primary screening, it was found that maximum number of isolates showed activity against *Bacillus cereus* (17.28%) followed by *Fusarium oxysporum* (14.11%), *Candida albicans* (11.26%), *Staphylococcus aureus* (2.65%) and *Escherichia coli* (2.24%). Isolates 196, 51 and 173 were selected for further analyses on the basis of their maximum and broad spectrum activity during primary screening. Activities of these cultures were quantified during secondary screening. It was revealed by bioautography that single bioactive fractions were present in ethyl acetate extracts of isolates 196 (R_f: 0.48) and 173 (R_f: 0.90) whereas the ethyl acetate extract of isolate 51 (R_f:0.90, 0.51) had two bioactive fractions. Bioactive fractions present in ethyl acetate extracts of isolates 196 and 173 showed activity against *Bacillus cereus* and *Fusarium oxysporum* respectively, whereas methanolic extract of isolate 51 showed broad spectrum activity.

KEYWORDS: Actinomycetes; bioactive compounds; ethyl acetate and methanolic extracts; antimicrobial analyses; bioautography

INTRODUCTION

Pathogenic microbes are becoming resistant to multiple drugs which has triggered the need for discovery and development of new antimicrobial compounds with altered modes of action. Various disease causing microbes such as *Clostridium difficile, Enterococcus faecium, Enterococcus faecalis, Mycobacterium tuberculosis, Neisseria gonorrhoeae, Pseudomonas aeruginosa, Salmonella enterica, Staphylococcus aureus* and *Streptococcus pneumoniae* have become resistant to antibiotics that are being used for their treatment [1-8]. Resistance was acquired by alteration of target sites, efflux of antibiotics resulting in their low intracellular concentration, production of enzymes which make the target drug inactive and reduced drug permeability [9]. As a result, new drugs need to be discovered to cure diseases caused by MDR strains.

Natural products from microbes are useful source of new drugs. Bacteria are well known for production of important commercial metabolites. Among bacteria, actinomycetes are well documented group for production of large number of antibiotics [10]. Antibiotics produced by actinomycetes belong to different chemical groups including tetracyclins, ß-lactams, macrolides, ansamycins, peptides to name a few and they possess a range of biological activities like antibacterial, antifungal, antitumor and insecticidal [11]. Among actinomycetes, *Streptomyces* genus is major producer of about 60% known antibiotics with some contribution from other genera [12]. Isolation of actinomycetes from diverse ecological habitats and their antimicrobial analyses is useful for search of novel antibiotics [10]. Isolates are subjected to preliminary or primary screening to check whether isolates have potential for production of any antimicrobial compounds. It is a qualitative approach. There are different methods for primary screening including Cross streak/Perpendicular streak method, Agar plug/Agar disk method, Spot inoculation method and Agar piece method [13]. Organic solvents of different polarities are used to recover bioactive compounds from culture broth or from culture media plates [14, 15]. Organic solvent fraction containing bioactive compounds are dried in a rotary evaporator under vacuum at 40ºC-45ºC. Secondary screening is done to quantify the activity of antimicrobial compounds either by paper disk activity method or by agar well diffusion/agar cup method [15, 16]. Bacteria produce a large number of secondary metabolites during fermentation process. It is required to identify actual bioactive fraction. This is done by Bioautography, which is simple, easy to perform and quite sensitive method [17, 18]. Fractions are subsequently purified either by preparative thin layer chromatography or by column chromatography.

METHODOLOGY

Isolation of bacteria

Soil samples were collected from diverse ecological habitat as shown in Table 1**. S**amples were dried for 24-48 hrs depending on their moisture content. Serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}) of dried soil samples were prepared in saline (0.9% NaCl). Dilutions were plated on actinomycete specific media including Yeast Extract Malt Extract agar, Glycerol asparagine agar, Arginine glycerol agar, Starch casein agar and Organic agar Gause and incubated at 28°C for 7-10 days[19, 20]. Colonies were purified by 2-3 rounds of streaking on YM plates and stored at -80°C as glycerol stocks for long term preservation.

Table 1: Soil samples were collected from diverse habitats as mentioned below

Sl.No.	Habitat	Total no. of colonies
1.	AGRICULTURAL SOILS	
	Agricultural soil, Dhanaura, U.P	86
	Agricultural soil, Yamuna	37
	Agricultural soil, Nainital	13
	Agricultural soil, Kashipur	17
2.	INDUSTRIAL SOILS	
	Sugar Plant, Dhanaura, U.P	7
	Chemical Plant, Faridabad	10
3.	LANDFILL SOILS	
	Dumping site, Sarai Kale Khan,	28
4.	RIVER/LAKE SOILS	
	Yamuna Bank	6
	Lake soil, Purana Quila	14
5.	DIVERSITY PARK SOILS	
	Diversity Park, Sarai Kale Khan	8
	Great Himalayan, National Park, Teerthan valley, H.P	42
	Great Himalayan, National Park near a narrow spring, Teerthan valley, H.P	38
6.	SEA/BEACH SOILS	
	Catamaran Beach Hotel, Colombo, Sri Lanka	20
	Havelock Islands, Andaman & Nicobar Islands	76
	Carbon Island, Andaman & Nicobar Islands	98

7.	FOREST SOILS	
	Killingpong 4000ft ,Kolkata	44
	Pine Forest, Teerthan valley, H.P	102
	Total number of isolates	646

ANTIMICROBIAL ANALYSES OF ISOLATES

Primary Screening

Actinomycete cultures were tested for production of antimicrobial compounds against the five pathogens *Bacillus cereus* MTCC 430, *Candida albicans* MTCC 227, *Escherichia coli* MTCC 443, *Fusarium oxysporum* MTCC 284 and *Staphylococcus aureus* MTCC 740.

Actinomycete cultures were streaked on one half of the YM plate and incubated at 28°C for 7-10 days. Sensitive strain seeded plates were prepared by mixing 5ml of overnight grown culture with 50ml of soft agar and over layered 3ml of suspension on growth media plates. After solidification of top layer, agar plugs were cut out from culture plates of different isolates and placed on a single sensitive strain seeded plate. Plates were incubated overnight at optimum temperatures. Inhibition zones around plugs after incubation period were observed for production of any antimicrobial compounds [13, 21]**.** Experiment was repeated with all the sensitive strains.

Secondary screening

Extraction of antimicrobial compounds from culture plates

Actinomycete colonies were inoculated on YM media plates and incubated for 7 days. Agar medium from 4-5 culture plates was cut into small pieces and collected in a flask having approximately 100ml solvent (Ethyl acetate or methanol). Extraction flask was kept on shaker for about 3-4hrs at high speed. Organic phase was separated by centrifugation and filtration. Organic solvent having bioactive components was evaporated in a rotavapor (BUCHI R-200) at 40ºC-45ºC to remove the solvent and powdered form of extracts were collected [22].

Secondary screening of isolates using agar well method

Sensitive strain seeded plates were prepared by mixing appropriate volume of log phase culture with 50ml of soft agar and over-layering 3ml of this suspension on to growth medium plates. Top layer was allowed to solidify for half an hour in the laminar flow hood. Wells of 5-7mm diameter were bored into the sensitive strain seeded agar plate. Different concentrations of solvent extract were added into the wells. Plates were incubated at appropriate temperature for 24-48 hours and diameter of the zones of inhibition produced by bioactive residues were measured. Simultaneously, activities of commercial antibiotics were determined by agar well method and compared with those of bacterial extracts [15, 23].

Thin layer chromatography of extracts

Appropriate volume of bioactive compound was applied to the TLC plate (Merck silica gel plate 60 F_{254}, 0.2mm). Spots were air dried. Chromatograms were developed either using Dichloromethane:Methanol (9:1) or Butanol:Acetic acid:Water (3:1:1) as the mobile solvent system. Spots were marked with the help of UV and their retention factor (R_f) values were calculated **[**23, 24].

Bioautography

Developed chromatograms of culture extracts were air dried and fractionated spots were observed and marked under UV at 365 nm. Soft agar was supplemented with log phase culture of sensitive strain having 10^5 cfu's/ml and spread over TLC plates. After overnight incubation at 37°C, TLC plates were stained with thiazolyl blue tetrazolium bromide (MTT tetrazolium) (1mg/ml in distilled water). Plates were observed for appearance of white inhibition zones (around marked fractions) against purple background after incubation at 30°C for 4 hours. An identical TLC plate on which extracts had been run was used to ascertain the position of actual bioactive fractions on the chromatogram [23].

RESULTS

Isolation of bacteria

A total of 646 bacterial colonies were isolated from soil samples collected from diverse ecological habitats including agricultural, industrial, landfill sites, river/lake sediments, Diversity Park, sea/beach sediments and forest sites. Amongst these, agricultural samples from

Dhanaura (U.P) gave the largest yield of actinomycete colonies with antibacterial activity. Among various media tested for bacterial isolation, arginine glycerol medium gave the maximum yield of actinomycetes (Figure 1).

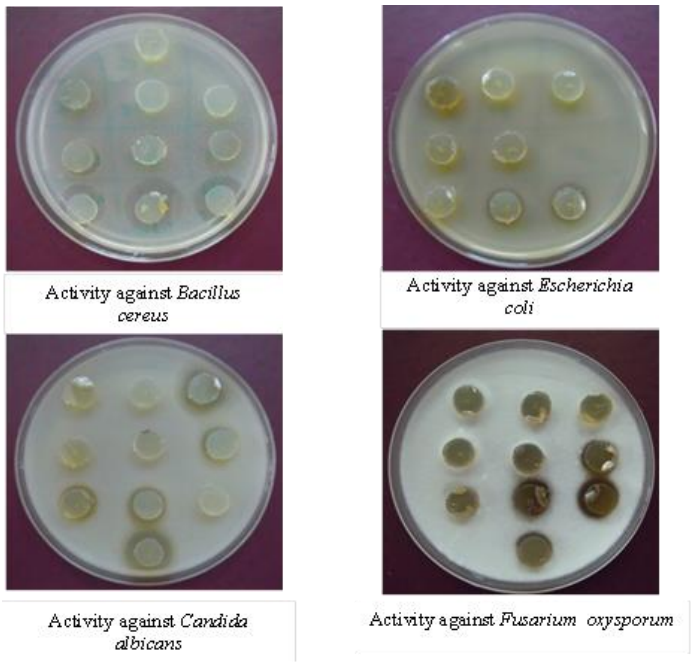


Fig. 1: Primary screening of isolates for antimicrobial activity. Pictures represent plugs taken from individual plates of isolates and placed on pathogen seeded plates. Zones of inhibition of variable diameter appear around the plugs after 24h incubation

Antimicrobial analyses of isolates

Primary Screening

Maximum number of isolates showed activity against *Bacillus cereus* (17.28%) followed by *Fusarium oxysporum* (14.11%), *Candida albicans* (11.26%), *Staphylococcus aureus* (2.65%) and *Escherichia coli* (2.24%) (Figure 2). Some isolates were active against more than one pathogen.

Isolates 196, 51 and 173 were finally selected for further analyses on basis of their high activity during primary screening (Table 2).

Table 2: Potent strains selected for secondary screening

S.No.	Isolates selected for secondary screening	Activity against pathogen
1	196	*Bacillus cereus*
2	212	*Staphylococcus aureus*
3	51	*Candida albicans, Bacillus cereus, Fusarium oxysporum*
4	22	*Escherichia coli*
5	173	*Fusarium oxysporum*

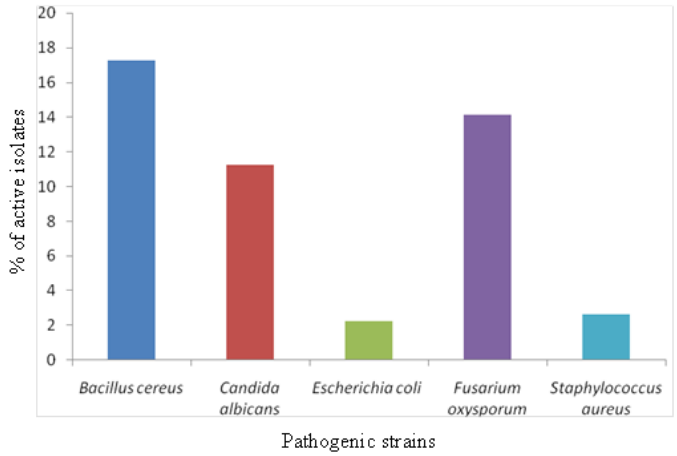


Figure 2: Percentage of isolates active against different pathogens

Secondary screening

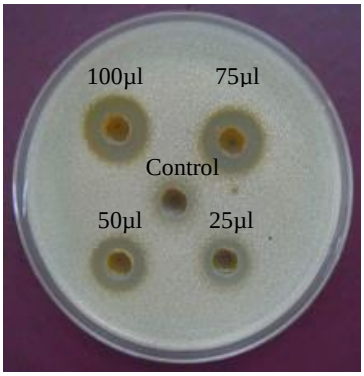
Extraction of antimicrobial compounds from culture plates

Bioactive compounds were extracted from media plates using solvents of variable polarity. Organic solvent extraction method was used for extraction of antimicrobial compounds from solid media plates using either ethyl acetate or methanol. Less polar metabolites could be extracted in less polar solvent like ethyl acetate and metabolites having high polarity could be extracted in methanol. This combination of solvents was helpful in extraction of both classes of metabolites from the bacteria.

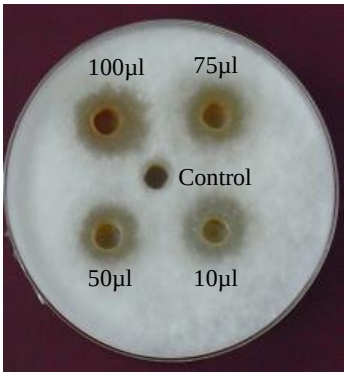
Secondary screening of isolates using agar well method

Different dilutions of extracts were prepared and their bioactivity was quantified using agar well method. Activity of extracts was compared with those of control antibiotics. Kanamycin acid sulphate, Amphotericin B and Cycloheximde were used as positive controls against *Bacillus cereus*, *Candida albicans* and *Fusarium oxysporum,* respectively (Tables 3, 4 and 5).

In most of the cases, high activity was observed in control antibiotics as compared to those of antimicrobial compounds extracted from culture broth of actinomycete isolates as depicted in Tables 3, 4 and 5 and Figures 3, 4 and 5. The main reason of this was that the actinomycete extracts were not pure leading to reduction of activity. Secondly, impurities in the extract may also hamper their diffusion in the agar plates and correspondingly lowered the activity (Figures 3, 4, 5 and 6).



(a) Activity of isolate 51 methanol extract against C*andida albicans*



(b) Activity of isolate 173 methanol extract against *Fusarium oxysporum*

Figure 3: **Secondary Screening**

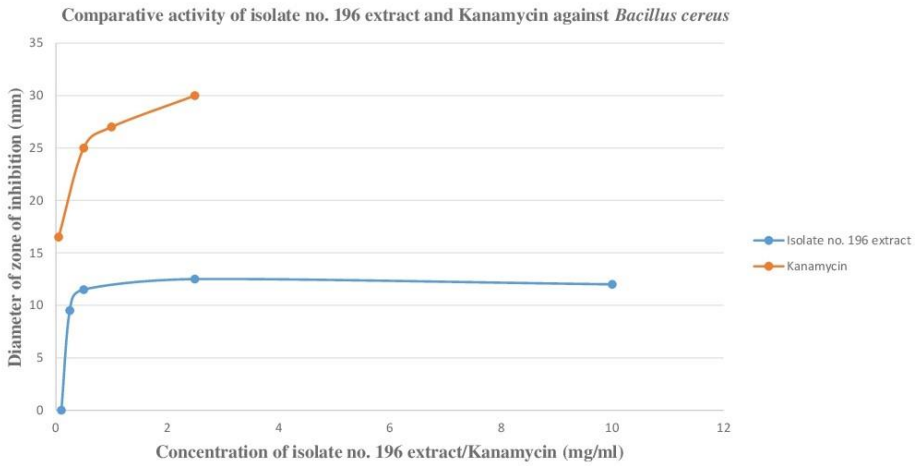


Fig. 4: Comparative activity of ethyl acetate extract of isolate no. 196 and Kanamycin against *Bacillus cereus*.

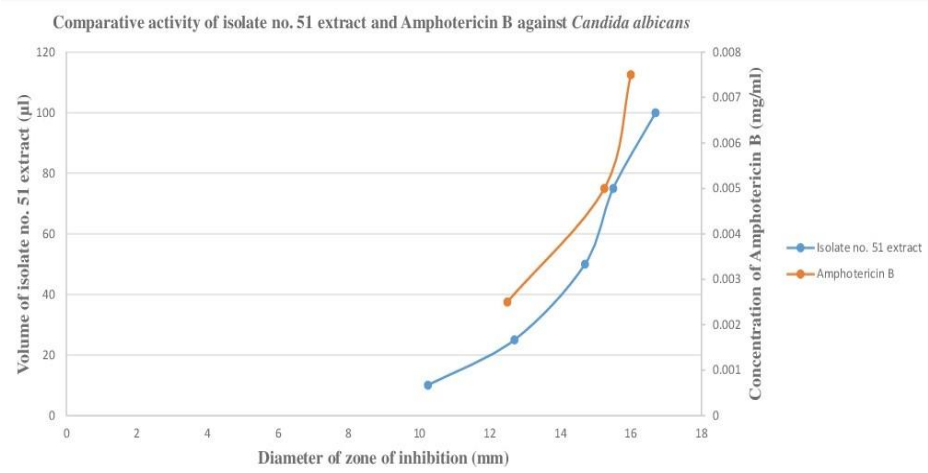


Fig. 5: Comparative activity of methanolic extract of isolate no. 51 and Amphotericin B against *Candida albicans*

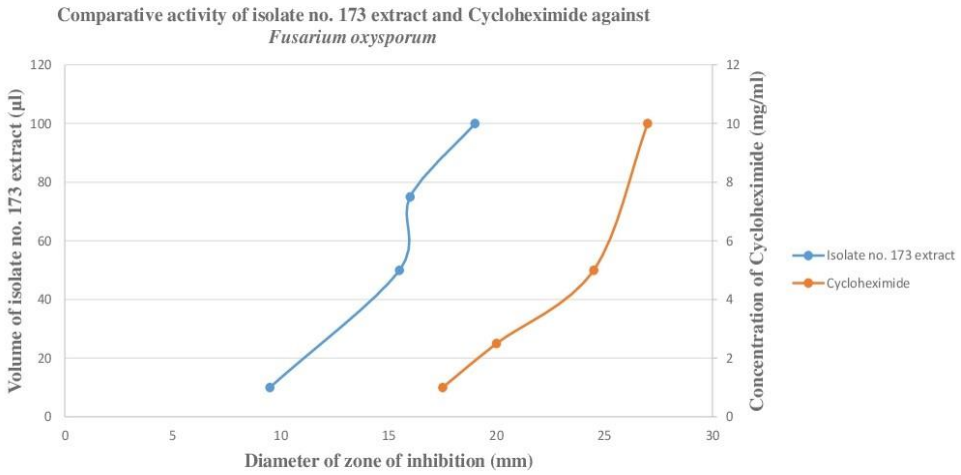
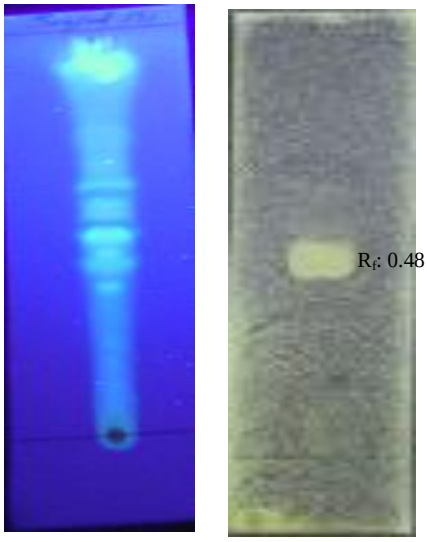


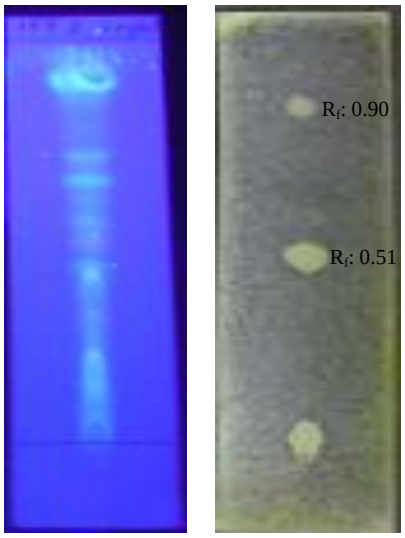
Fig. 6: Comparative activity of methanolic extract of isolate no. 173 and Cycloheximide against *Fusarium oxysporum*

Thin layer chromatography of extracts and Bio autography

Actual bioactive fractions were determined by bioautography. Ethyl acetate extracts of isolates 196 (R_f: 0.48) and 173 (R_f: 0.90), showed a single bioactive fraction active against *Bacillus cereus* and *Fusarium oxysporum*, respectively. Single bioactive fraction from 51 methanolic extract (R_f: 0.62) showed activity against both *Candida albicans* and *Fusarium oxysporum* indicating its broad spectrum antimicrobial activities. Two bioactive fractions were present in 51 ethyl acetate extract (R_f : 0.90, 0.51) active against *Bacillus cereus* (Figure 7).



TLC profile and bioactive fraction of isolate 196



TLC profile and bioactive fractions of isolate 51

Fig.7: Thin Layer Chromatography and Bioautography

Table 3: Activity of ethyl acetate extract of isolate 196 and Kanamycin against *Bacillus cereus*

Sl.No.	Concentration of extract (mg/ml)	Diameter of inhibition zones (in mm)	Concentration of Kanamycin (mg/ml)	Diameter of inhibition zones (in mm)
1.	10	12	2.5	30
2.	2.5	12.5	1	27
3.	0.5	11.5	0.5	25
4.	0.25	9.5	0.05	16.5
5.	0.1	No inhibition zone		

Table 4: Activity of methanol extract of isolate 51 and amphotericin B against *Candida albicans*

Sl.No.	Volume of extract (µl)	Diameter of inhibition zones (in mm)	Concentration of Amphotericin B (mg/ml)	Diameter of inhibition zones (in mm)
1.	100	16.7	0.0075	16
2.	75	15.5	0.0050	15.25
3.	50	14.7	0.0025	12.5
4.	25	12.7		
5.	10	10.25		

Table 5: Activity of methanol extract of isolate 173 and cycloheximide against *Fusarium oxysporum*

Sl.No.	Volume of extract (µl)	Diameter of inhibition zones (in mm)	Concentration of cycloheximide (mg/ml)	Diameter of inhibition zones (in mm)
1.	100	19	10	27
2.	75	16	5	24.5
3.	50	15.5	2.5	20
4.	10	9.5	1	17.5

DISCUSSION

Actinomycetes were isolated from diverse ecological habitats. The logic behind this is that actimomycetes from a range of habitats would have variable metabolic profiles and may be capable of producing other new antimicrobial compounds or derivatives of existing compounds. Soil samples were taken from the top layer as with the increase of depth, moisture content and organic matter decreases which hampers the growth and existence of microbes such as bacteria and fungi in soil [25-27].

Purified actinomycetes were subjected to antimicrobial analyses. It was found that 17.28%, 14.11%, 11.26%, 2.65% and 2.24% isolates showed activity against *Bacillus cereus, Fusarium oxysporum, Candida albicans*, *Staphylococcus aureus* and *Escherichia coli*. This preponderance of activity of isolates against gram positive bacteria may be due to morphological differences between Gram positive and negative bacteria. Gram negative bacteria are less permeable as compared to Gram positive bacteria [28]. Therefore, maximum activity was observed against Gram positive bacteria [29, 30].

Antimicrobial compounds were extracted using a combination of ethyl acetate and methanol that differ in their respective polarity. As a result of this combination of solvents hydrophobic antimicrobial compounds could be extracted in ethyl acetate whereas hydrophilic compounds could be extracted with methanol [18].

Activity of extracts was quantified during secondary screening using agar well method and compared with those of control antibiotics. In most of the cases control antibiotics showed more activity as compared to antimicrobial compounds extracted from culture broth of actinomycete isolates possibly because the extracts were not pure resulting in lowered activity or impurities might hamper the diffusion of extracts in adjacent medium [31].

During extraction of antimicrobial compounds a large number of secondary metabolites are produced [24]. Therefore, it is required to identify actual bioactive fraction. Actual bioactive fractions were identified by bioautography. Extract from culture no. 196 and 173 showed activity against one pathogenic strain whereas methanolic extract from culture no. 51 showed activity against two sensitive strains indicating its broad antimicrobial spectrum.

CONCLUSION

Actinomycetes were isolated from diverse ecological habitats. Isolates from agricultural soil showed maximum activity as compared to other habitats. Actinomycetes showed high activity against Gram positive bacteria as compared to Gram negative bacteria, fungi and yeast. Isolates were selected for further analyses on the basis of their broad spectrum activities. Activities of extracts were quantified and compared with those of control antibiotics. Further this work can be extended to structure elucidation of compounds and identification of gene clusters responsible for production of these antimicrobial compounds. Potent strains can be characterized taxonomically on the basis of polyphasic approach including genotypic, phenotypic and chemotypic studies.

ACKNOWLEDGMENTS

This work was supported by grants from University of Delhi under Innovation Project scheme. Infrastructural facilities provided by Acharya Narendra Dev College (ANDC), University of Delhi are gratefully acknowledged.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest in this research article.

REFERENCES

1. Peng Z, Jin D, Kim HB, Stratton CW, Wu B, Tang Y, Sun X. Update on Antimicrobial Resistance in *Clostridium difficile*: Resistance Mechanisms and Antimicrobial Susceptibility Testing. J Clin Microbiol 2017; 55(7): 1998-2008.
2. Miller WR, Munita JM, Arias CA. Mechanisms of antibiotic resistance in *enterococci*. Expert Rev Anti-infect Ther 2014; 12(10): 1221-1236.
3. Nguyen L. Antibiotic resistance mechanisms in *M. tuberculosis*: an update. Arch Toxicol 2016; 90: 1585-1604.
4. Costa-Lourenco APR, Santos KTB, Moreira BM, Fracalanzza SEL, Bonelli RR. Antimicrobial resistance in *Neisseria gonorrhoeae*: history, molecular mechanisms and epidemiological aspects of an emerging global threat. Braz J Microbiol 2017; 48(4): 617-628.
5. Lambert PA. Mechanisms of antibiotic resistance in *Pseudomonas aeruginosa*. J Royal Soc Med 2002; 95: 22-26.
6. Spengler G, Rodrigues L, Martins A, Martins M, McCusker M, Cercaa P, Machadoa L, Costaa SS, Ntokoue E, Coutoa I, Viveiros M, Fanning S, Molnar J, Amaral L. Genetic response of *Salmonella enterica* serotype Enteritidis to thioridazine rendering the organism resistant to the agent. Int J Antimicrob Agents 2012; 39: 16-21
7. Foster TJ. Antibiotic resistance in *Staphylococcus aureus*. Current status and future prospects. FEMS Microbiol Rev 2017; 41(3): 430-449.
8. Moujaber GE, Osman M, Rafei R, Dabboussi F, Hamze M. Molecular mechanisms and epidemiology of resistance in *Streptococcus pneumoniae* in the Middle East region. J Med Microbiol 2017; 66: 847-858.
9. O'Connor JR, Galang MA, Sambol SP, Hecht DW, Vedantam G, Gerding DN, Johnson S. Rifampin and rifaximin resistance in clinical isolates of *Clostridium difficile*. Antimicrob Agents Chemother 2008; 52: 2813-2817.
10. Genilloud O. Actinomycetes: still a source of novel antibiotics. Nat Prod Rep 2017; 34: 1203-1232.
11. Mahajan GB, Balachandran L. Antibacterial agents from actinomycetes - A Review. Front Biosci 2012; 4: 240-253.
12. Esnault C, Dulermo T, Smirnov A, Askora A, David M, Deniset-Besseau A, Holland I, Virolle M. Strong antibiotic production is correlated with highly active oxidative metabolism in *Streptomyces coelicolor* M145. Sci Rep 2017; 7: 200.
13. John G Holt. Bergey's manual of systemic bacteriology. UK: Cambridge University Press; 1989, p 4.
14. Selvin J, Shanmughapriya S, Gandhimathi R, Seghal KG, Rajeetha RT, Natarajaseenivasan K, Hema TA. Optimization and production of novel antimicrobial agents from sponge associated marine actinomycetes *Nocardiopsis dassonvillei* MAD08. Appl Microbiol Biotechnol 2009; 83: 435-445.
15. Chaudhary HS, Yadav J, Shrivastava AR, Singh S, Singh AK, Gopalan N. Antibacterial activity of actinomycetes isolated from different samples of Sheopur (A city of central India). J Adv Pharm Technol Res 2013; 4: 118-123.
16. Mohseni M, Norouzi H, Hamedi J, Roohi A. Screening of antibacterial producing actinomycetes from sediments of the Caspian Sea. Int J Mol Cell Med 2013; 2: 64-71.
17. Tanvir R, Sajid I, Hasnain S, Kulik A, Grond S. Rare actinomycetes *Nocardia caishijiensis* and *Pseudonocardia carboxydivorans* as endophytes, their bioactivity and metabolites evaluation. Microbiol Res 2016; 185: 22-35.
18. Khanna M, Solanki R, Lal R. Selective isolation of rare actinomycetes producing novel antimicrobial compounds. Int J Adv Biotechnol Res 2011; 2: 357-375.
19. Saadoun I, Hameed KM, Moussauui A. Characterization and analysis of antibiotic activity of some aquatic actinomycetes. Microbios 1999; 99:173-179.
20. Solanki R, Lal R, Khanna M. Antimicrobial activities of actinomycetes from diverse ecological habitats in Delhi and its adjoining States, India. J Microb World 2011; 13: 233-240.
21. Xie ZP, Xu ZN, Shen WH, Cen PL. Bioassay of mildiomycin and a rapid cost effective

agar plug method for screening high yielding mutants of mildiomycin. World J Microbiol Biotechnol 2005; 21: 1433-1437.
22. Schimana J, Fiedler HP, Groth I, Süssmuth R, Beil W, Walker M, Zeeck A. Simocyclinones, novel cytostatic angucyclinone antibiotics produced by *Streptomyces antibioticus* Tü 6040. I. Taxonomy, fermentation, isolation and biological activities. J Antibiot 2000; 53: 779-787.
23. Selvameenal L, Radhakrishnan M, Balagurunathan R. Antibiotic Pigment from desert soil actinomycetes; biological activity, purification and chemical screening. Indian J Pharm Sci 2009; 71: 499-504.
24. Solanki R, Das P, Khanna M. Metabolic profiling of actinomycetes having antimicrobial properties. Int J Adv Biotechnol Res 2013; 4: 444-459.
25. Corke CT, Chase FE. Comparative studies of actinomycete populations in acid podzolic and neutral mull forest soils. Soil Sci Soc Am J 1964; 28: 68-70.
26. Essien JP, Udosen ED. Distribution of actinomycetes in oil contaminated ultisols of the Niger Delta (Nigeria). J Environ Sci 2000; 12: 296-302.
27. Paul NW, Mwaura FB, Wagacha JM, Gathuru EM. Methods of Isolating Actinomycetes from the Soils of Menengai Crater in Kenya. AC Microb 2017; 8(3):1-7.
28. Scherrer R, Gerhardt P. Molecular sieving by the *Bacillum megaterium* cell wall and protoplast. J Bacteriol 1971; 107: 718-735.
29. Anansiriwattana W, Tanasupawat S, Amnuoypol S, Suwanborirux, K. Identification and antimicrobial activities of actinomycetes from soils in Samed Island, and geldanamycin from strain PC4-3. Thai J Pharm Sci 2006; 30: 49-56.
30. Charoensopharat K, Thummabenjapone P, Sirithorn P, Thammasirirak S. Antibacterial substance produced by *Streptomyces* sp. No. 87. Afr J Biotechnol 2008; 7: 1362-1368.
31. Thenmozhi M, Kannabiran K. Studies on isolation, classification and phylogenetic characterization of novel antifungal *Streptomyces* sp. VITSTK7 in India. Curr Res J Biol Sci 2010; 2: 306-312.

Cite this article as:
Monisha Khanna Kapur, Renu Solanki, Payal Das, Munendra Kumar, Prateek Kumar. Antimicrobial Activity Analysis of Bioactive Compounds from Soil Actinomycetes. J Pharm Chem Biol Sci 2018; 6(3):178-187.