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Research Article

Antimicrobial Activity of Actinomycetes Against Multidrug Resistant *Staphylococcus aureus*, *E. coli and* Various Other Pathogens

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Abstract

Purpose: *The rapid emergence of drug resistance among pathogenic bacteria, especially multidrug-resistant bacteria, underlines the need to look for new antibiotics.*
Methods: *In the present study, 134 different actinomycetes, isolated from the soil samples collected from different localities of Punjab and Himachal Pradesh, were screened for antimicrobial activity against various test organisms including multidrug-resistant methicillin-resistant Staphylococcus aureus (MRSA) and Escherichia coli in order to identify potential antibiotic producers.*
Results: *Among these isolates, 51 (38 %) showed antimicrobial activity against one or more test organisms and six exhibited promising broad-spectrum activity against all the tested organisms. The observed cultural, morphological, physiological and biochemical characteristics confirmed that these isolates are species of the genus, Streptomyces.*
Conclusion: *Further studies on the bioactive metabolites from these cultures will be useful for discovering novel compounds of clinical and agricultural use.*

Keywords*: Actinomycetes, Broad spectrum antibiotics, Multidrug-resistant Staphylococcus aureus, Streptomyces.*

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INTRODUCTION

Since man started suffering from diseases caused by infectious microorganisms, the quest for their remedies started and led to the discovery of a large number of antibiotics from microorganisms including actinomycetes. Microorganisms have made an outstanding contribution to the health and well-being of people throughout the world [1]. Actinomycetes are filamentous gram-positive bacteria with high G + C content and are the most widely distributed group of microorganisms in nature which primarily inhabit the soil [2-3]. Among actinomycetes, the genus *Streptomyces* has long been recognized as a rich source of useful secondary metabolites and continues to be a major source of new bioactive molecules [4-6]. They are the origin of a good number of marketed antibiotics [7].

However, rapid emergence of antimicrobial resistance among pathogenic microorganisms has led to a renewed search for new antimicrobial agents from *Streptomyces*. Severe infections caused by bacteria that are resistant to commonly used antibiotics have become a major global healthcare problem in the 21st century [8]. The most resistant bacteria causing important community-acquired infections include methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Staphyloco-ccus aureus* (VRSA), vancomycin-resistant *Enterococcus* (VRE), extended spectrum β-lactamase (ESBL) producing bacteria such as *E. coli* and *Klebsiella* spp and multiple drug resistant *Mycobacterium tuberculosis* (MDR-MTB). Therefore, it is imperative to search for new, efficacious and safe antibiotics from natural sources to combat the menace of drug-resistant infections.

In view of the foregoing, the objective of the present investigation was to screen soil samples collected from different localities of Punjab and Himachal Pradesh, which are large, diverse and largely unscreened ecosystems, for the isolation of potent and broad-spectrum antibiotic-producing actinomycetes.

EXPERIMENTAL

Isolation of actinomycetes

The soil samples from different localities of Punjab and Himachal Pradesh (India) were collected in sterile polyethylene bags, sealed tightly and stored in a refrigerator. Soil samples were given three different pretreatments: dry heat (heating at 100 ºC for 1 h), wet heat (heating at 70 ºC in a water bath for 15 min) and 1.5 % phenol treatment. Each pretreated soil sample was serially diluted to 10^{-6} – fold and then 0.1 ml aliquot from each dilution was spread on starch-casein medium containing (g/l): starch 10.0, casein 0.3, KNO_3 2.0, NaCl 2.0, K_2HPO_4 2.0, $MgSO_4.7H_2O$ 0.05, $CaCO_3$ 0.02, $FeSO_4.7H_2O$ 0.01 and agar 18.0, which was supplemented with cycloheximide (50 µg/ml) and nystatin (25 µg/ml, HiMedia). The plates were incubated at 28 ºC for 28 days. The colonies of actinomycetes were recognized according to their rough and chalky macroscopic characteristics, and were then purified, transferred to starch casein nitrate agar slants (without any antifungal and antibacterial) and preserved at 4 ℃. The isolates were maintained as spore suspensions and mycelia fragments in 20 %v/v glycerol at -70 ºC in an ultra-low temperature freezer.

Test organisms

Various test organisms used in this study include: *Bacillus megaterium* (MTCC 428), *Bacillus subtilis* (MTCC 619), *Enterobacter aerogenes* (MTCC 111), *Escherichia coli* (MTCC 1885), *Klebsiella pneumoniae* sub sp. *pneumoniae* (MTCC 109), *Proteus mirabilis* (MTCC 1429), *Salmonella typhi* (MTCC 733), *Candida albicans* (MTCC 3017), *Rhodotorula rubra* (MTCC 248); clinical isolates (provided by Postgraduate Institute of Medical Sciences, Chandigarh, India) *Enterococcus* sp., two multidrug resistant *E. coli* strains

(S1-LF and R65-LF, both resistant to cephotaxime, ciprofloxacin, rifampicin, clindamycin and cefoperzone; R65-LF is also resistant to trimethoprim) and three methicillin-resistant *Staphylococcus aureus* strains (MRSA P-169, MRSA C-67 and MRSA C-97, which are also resistant to erythromycin, cefepime, gentamicin, co-trimoxazole, netilimycin, amikacin and imipenem).

Antimicrobial screening and selection of isolates

Primary screening was carried out using the modified method of Kirby Bauer antibiotic susceptibility test [9]. Antibiotic activity was determined on Mueller Hinton agar and Yeast malt agar media (Hi-Media) inoculated with bacteria and yeasts, respectively. The actinomycetes isolates were lawn-cultured by dense streaking on starch casein nitrate medium plates and incubated at 30 ºC for seven days. Six mm agar discs were prepared using sterile cork borer from well-grown culture and placed on fresh lawn culture of test organisms. The plates were then kept at 4 ºC for 30 min for the diffusion of the culture broth, and then incubated at their respective optimum temperature (37 ºC for bacteria and 25 ºC for the yeasts). The zones of inhibition for bacteria were determined after 18 - 24 h and for yeasts after 2 -3 days.

Isolates which showed broad spectrum activity against test organisms in primary screening were subjected to secondary screening by Kirby Bauer agar well diffusion method. Erlenmeyer flasks (250 ml) containing 50 ml of starch casein nitrate broth were inoculated with 7 days old culture and incubated at 28 ℃ for 5 days at 180 rpm. The culture broth was centrifuged and the activity of the supernatant was determined against test organisms by adding 50 µl to wells (6mm) bored into freshly inoculated plates. The plates were then kept at 4 ℃ for 30 min for diffusion of the antibiotic, they were then incubated at their respective optimum temperature and zone of inhibition was recorded.

Extraction of antibiotic

For the isolation of antimicrobial compound, five days old culture broths were extracted twice with six different solvents of a wide range of polarity, namely, n-butanol, chloroform, diethyl ether, ethyl acetate, hexane: methanol (20:50) and hexane. The solvent extracts were concentrated to dryness using rotavapour and tested for their antimicrobial activity against various test organisms.

Characterization of selected isolates using polyphasic approaches Morphological, cultural, physiological and biochemical characterization

The six selected isolates (2A, A26, A27, A13, N23 and R3YS which showed promising broad spectrum activity) were characterized morphologically and physiologically according to International *Streptomyces* Project [10-11]. The morphological characteristics were examined by culturing isolates on different ISP media (ISP-2, ISP-3, ISP-4, ISP-5, ISP-7) and Bennett's agar. The plates were incubated at 28 ºC for one week. Physiological tests - growth at various temperatures (20 – 37 ºC) and NaCl concentrations (2 - 10 %) were examined by growing the strains on starch casein nitrate (SCN) agar medium. Starch hydrolysis was investigated after 7 days on starch casein nitrate agar by flooding the plates with a 1 %w/v iodine solution [12]. To check esculin hydrolysis, isolates were grown on esculin hydrolysis medium for 7 days and observed for appearance of dark brown to black coloration [12]. Gelatin hydrolysis was tested after growing isolates for 7 days on gelatin containing medium. To examine for hydrolysis, culture tubes were chilled in ice water and checked for solidification. Hydrolysed gelatin remained fluid [12]. For nitrate reduction, isolates were grown in

nitrate containing medium for a week and thereafter, nitrate reduction was investigated by addition of Griess- Illosvay's reagent [13]. For urea hydrolysis, 2 % urea was added to basal medium. After a week, change in color from yellow to red was recorded [14]. Assimilation of sugars as carbon sources and acid production were studied by adding 1 % filter-sterilized sugars to the basal medium (ISP-9). D-glucose containing medium was considered as a positive control while medium without sugar was negative control. Growth on other sugars (sucrose, xylose, inositol, D-Mannitol, D-fructose, L-Rhamnose, D-raffinose and cellulose) was compared to positive control after 2 weeks of incubation [10].

Chemotaxonomic characterization

Analysis of the isomer of diaminopimelic acid in the cell wall and sugars in the whole-cell hydrolysate was done according to Kutzner [15]. For sugar determination, dried cells (50 mg) were hydrolyzed in 1 ml of H_2SO_4 at 100 ℃ for 2 h and neutralized with saturated solution of $Ba(OH)_2$. The supernatant was dried, dissolved in distilled water and spotted (5µl) on a thin-layer plate coated with silica gel along with reference sugars (xylose, galactose, arabinose and glucose 5mg/ml each). For the determination of diaminopimelic acid (DAP), dried cells (1 mg) were hydrolyzed with 6M HCl at 100 ℃ for 18 h. The sample was dried and dissolved in distil water and spotted (5 µl) on thin layer plate along with 2 µl DAP acid (0.01M) as reference standard.

Statistical analysis

To analyze the antimicrobial activity of the different actinomycete isolates against the various test organisms, the data (expressed as the mean ± standard error of mean (SEM) of three replicates) were subjected to two-way analysis of variance (ANOVA) and the means were compared using Tukey's HSD at a significance level of $p < 0.05$ using Sigma Stat 3.5.

RESULTS

In this isolation and screening programme, 134 different actinomycete isolates (based on colony morphology) were obtained from the soil samples collected. Out of these, 51 isolates (38.0 %) exhibited antimicrobial activity during primary screening. All 51 isolates exhibited antibacterial activity against *B. megaterium* while only three (2.23 %) exhibited antiyeast activity (against *C. albicans* and *R. rubra*). The isolates exhibiting antimicrobial activity in primary screening were subjected to secondary screening by agar well method to confirm their activity in culture broth. Forty four isolates exhibited antimicrobial activity (32.8 %) in culture broth.

Six isolates - 2A, R3YS, N23, A26, A27 and A13 - showing promising broad spectrum activity against different test organisms were selected for further study. All the isolates when grown on starch casein nitrate broth showed maximal antibiotic production on the fifth day (Table 1). R3YS showed maximum inhibition zone against all the tested organisms followed by 2A isolate. Statistical analysis of the antimicrobial activity of actinomycete isolates against various test organisms showed significant interaction between actinomycete isolates and test organisms (Table 1).

Extraction of antibiotic

The antimicrobial compounds from the culture filtrates of the isolates 2A, R3YS, N23, A27 and A13 were extractable in n-butanol (1: 2 v/v) whereas from A26 was extracted in diethyl ether. This suggests the polar nature of antimicrobial substances from the culture filtrates of 2A, R3YS, A27, A13 and N23 while non polar nature of compound from A26.

Table 1: Antimicrobial activity of actinomycete isolates

Test organism	Zone of inhibition (mm)					
	2A	*R3YS*	*N23*	*A26*	*A27*	*A13*
MRSA-97	21.6 ± 0.5^{abu}	24 ± 1.0^{av}	17 ± 1.0^{aw}	18.3 ± 0.5^{acx}	17 ± 1.0^{aw}	17 ± 0.0^{aw}
MRSA-67	19.3 ± 0.5^{acfu}	21 ± 0.5^{bv}	14.6 ± 0.5^{bw}	15.6 ± 0.5^{bfwx}	15.6 ± 0.5^{abwx}	16 ± 1.0^{abx}
MRSA P-169	19.3 ± 0.5^{acfu}	24 ± 0.0^{av}	15 ± 0.5^{bwx}	15.6 ± 0.5^{befw}	15 ± 0.0^{bwx}	14.6 ± 0.5^{bcx}
B. megaterium	21.6 ± 0.5^{bgu}	23.3 ± 0.5^{adv}	20.3 ± 0.5^{cw}	19.3 ± 0.8^{cw}	17.3 ± 0.5^{ax}	16 ± 0.0^{acy}
B. subtilis	21.0 ± 1.0^{cfu}	20.3 ± 0.5^{bev}	17.3 ± 0.5^{aw}	17.6 ± 0.5^{adw}	14.6 ± 0.5^{bcx}	14.6 ± 0.5^{bcx}
R-6SLF	19.3 ± 0.5^{acfu}	19.3 ± 0.5^{cev}	10 ± 0.0^{dw}	14.6 ± 1.0^{eiw}	15 ± 0.0^{bx}	0^{dx}
SI-LF	12.6 ± 0.5^{du}	22.3 ± 0.5^{bdv}	14.3 ± 0.5^{bw}	16.3 ± 0.5^{bdx}	15 ± 1.0^{bw}	0^{dy}
E. coli	17.6 ± 0.5^{cefu}	20.3 ± 0.5^{bev}	14.6 ± 0.5^{bw}	16 ± 0.0^{bfxy}	15 ± 1.0^{bwx}	16.3 ± 0.5^{ay}
S. typhi	19 ± 1.0^{fu}	24.6 ± 0.8^{av}	18.0 ± 0.0^{au}	14.6 ± 0.5^{efiw}	13 ± 0.0^{dx}	0^{dy}
P. mirabilis	12.3 ± 0.5^{du}	15 ± 0.0^{gv}	0^{w}	11.6 ± 0.5^{gx}	0^{ew}	0^{dw}
K. pneumoniae	11.3 ± 0.5^{dhu}	12.6 ± 0.5^{fhkv}	9.3 ± 0.5^{dw}	9.3 ± 0.5^{gw}	10 ± 0.0^{fw}	10 ± 0.0^{ew}
Enterococcus	10 ± 1.5^{ijhu}	10.6 ± 0.5^{iu}	0^{ew}	10 ± 0.0^{gu}	0^{ew}	0^{dw}
E. aerogenes	16.3 ± 0.5^{eu}	17.3 ± 0.5^{ju}	15 ± 0.5^{bw}	12.3 ± 0.5^{hx}	13.3 ± 0.5^{cdx}	13.3 ± 0.5^{cfx}
R. rubra	16.3 ± 0.5^{eu}	19 ± 0.5^{cev}	0^{ew}	14 ± 0.0^{ix}	0^{ew}	0^{dw}
C. albicans	14.6 ± 0.5^{ku}	13.6 ± 0.5^{gku}	0^{ev}	11.6 ± 0.5^{ghw}	0^{ev}	0^{dv}
P. syringe	9.6 ± 0.5^{iu}	12 ± 0.0^{ifv}	0^{ew}	0^{jw}	0^{ew}	0^{dw}
M. smegmatis	23 ± 1.0^{gu}	25 ± 1.0^{av}	20.3 ± 0.5^{cw}	0^{jx}	0^{ex}	0^{dx}
X. campestris	0^{ju}	18.3 ± 0.5^{cv}	12.6 ± 0.5^{fw}	0^{ju}	0^{eu}	0^{du}

Note: *Results are mean ± SEM of three independent experiments. The same letters (a, b, c, d, e, f, g, h, i, j, k) within a row are not significantly different (Tukey's HSD, $p < 0.05$). The effect of different actinomycete isolates on antimicrobial activity. The same letters (u, v, w, x, y) within a column are not significantly different (Tukey's HSD, $p < 0.05$) and signify the effect of various test organism on antimicrobial activity of actinomycete isolates*

Characterization of selected isolates using polyphasic approaches: Morphological, cultural, physiological and biochemical characterization

All the isolates formed stable aerial and substrate mycelia when examined under phase contrast microscopy (slide culturing technique). The spore chain morphology of 2A and A26 belongs to straight chain section, while in R3YS, N23, A27 and A13, most of the spore chains were rectus flexibilis, and very few formed loops on the tips of the spore chain. The mature spores of A26 and A13 were round in shape and the number of spores varied from 25 - 45 per spore chain. However, in R3YS, A27, N23 and 2A, spores were oval and there were more than 20 per spore chain.

Cultural characteristics of isolates on different media are presented in Table 2. They grew well on most of the organic and synthetic media but the best growth of all the cultures was observed on starch casein nitrate agar. No soluble pigment was produced by 2A, R3YS, N23, A26 and A27 on any of the media used. However, isolate A13 produced yellow-colored soluble pigment in all the tested media except glycerol asparagine agar and tyrosine agar.

The various physiological characteristics of the isolates are presented in Table 3. All the isolates grew over a temperature range of 20 - 37 ºC with the optimum conditions at 28 ºC and 2 % NaCl concentration. All the isolates were capable of hydrolyzing starch and esculin but not gelatin (only N23 and A27 hydrolyzed gelatin). R3YS, N23 and A27

Table 2: Cultural characteristics of active isolates on different media

Medium	Charac-terstic	Isolate					
		2A	*R3YS*	*N23*	*A26*	*A27*	*A13*
Starch casein nitrate agar	G[a]	Abundant	Adundant	Abundant	Abundant	Abundant	Abundant
	AM[b]	Pinkish white	Peach	Whitish green	Pinkish white	Whitish green	Grey
	SM[c]	White	Light peach	Light yellow	Pinkish white	Whitish green	Grey
	SP[d]	None	None	None	None	None	Mustard
	S[e]	Pinkish white	Light peach	Pastal green	Pinkish white	Whitish green	Grey
Yeast extract malt extract agar (ISP2)	G	Abundant	Adundant	Abundant	Abundant	Abundant	Abundant
	AM	White	Light peach	Whitish green	White	Whitish green	Dark Grey
	SM	White	Peach	Cream	White	Whitish green	Grey
	SP	None	None	None	None	None	Mustard
	S	White	Light peach	Pastal green	White	Whitish green	Dark Grey
Oat meal agar (ISP3)	G	Abundant	Abundant	Abundant	Abundant	Abundant	Abundant
	AM	Pinkish white	Grey	Pastal green	Pinkish white	Whitish green	Grey
	SM	White	Grey	Cream	White	Whitish green	Grey
	SP	None	None	None	None	None	Mustard
	S	Pinkish white	Grey	Pastal green	Pinkish white	Pastal green	Grey
Inorganic salts agar (ISP4)	G	Abundant	Adundant	Abundant	Abundant	Abundant	Good
	AM	Pinkish white	Peach	Whitish green	Pinkish white	Whitish green	Yellow
	SM	Pinkish white	Peach	Cream	Pinkish white	Whitish green	Light yellow
	SP	None	None	None	None	None	Light yellow
	S	Pinkish white	Peach	Greenish	Pinkish white	Pastal green	Light yellow
Glycerol asparagine agar (ISP5)	G	Poor	Abundant	Abundant	Poor	Good	Abundant
	AM	White	White	Whitish green	White	Pastal green	Creamish
	SM	White	White	Cream	White	Pastal green	Creamish
	SP	None	None	None	None	None	None
	S	White	White	Whitish green	White	Whitish green	Whitish grey
Tyrosine agar (ISP7)	G	Abundant	Adundant	Abundant	Abundant	Abundant	Good
	AM	Pinkish white	Peach	Whitish green	Pinkish white	Pastal green	Grey
	SM	White	Light peach	Cream	White	Pastal green	Grey
	SP	None	None	None	None	None	None
	S	Pinkish white	Peach	Pastal green	Pinkish white	Whitish green	Grey
Bennett's agar	G	Abundant	Abundant	Abundant	Abundant	Abundant	Good
	AM	White	Grey	Whitish green	White	Whitish green	Light yellow
	SM	White	Grey	Cream	White	Whitish green	Light yellow
	SP	None	None	None	None	None	Light yellow
	S	White	Grey	Pastal green	White	Whitish green	Light yellow

[a]G = Growth, [b]AM = Aerial mycelium, [c]SM = Substrate mycelium, [d]SP = Soluble pigment, [e]S = Sporulation

reduced nitrate to nitrite and hydrolyzed urea. None of the isolates produced melanin on tyrosine agar. Assimilation of sugars as carbon sources and acid production by isolates were studied and the results are shown in Table 4.

Chemotaxonomic characterization

All the six isolates possess LL-DAP and glycine in their cell wall but no characteristic sugar. Phenotypic characteristics and chemotaxonomic characteristics indicated that all six active isolates belonged to the genus *Streptomyces*.

DISCUSSION

Actinomycetes are the most biotechnologically valuable prokaryotes responsible for the production of about half of the discovered bioactive secondary metabolites including antibiotics [3,4,6]. They

Table 3: Physiological characteristics of isolates

Characteristic	Medium	Response of isolates					
		2A	*R3YS*	*N23*	*A26*	*A27*	*A13*
Hydrolysis of starch	Starch casein nitrate agar	+	+	+	+	+	+
Hydrolysis of urea	Urea broth	-	+	+	-	+	-
Reduction of nitrate	Nitrate broth	-	+	+	-	+	-
Melanin formation	Tyrosine agar	-	-	-	-	-	-
Hydrolysis of esculin	Esculin hydrolysis medium	+	+	+	+	+	+
Hydrolysis of gelatin	Gelatin hydrolysis medium	-	-	+	-	+	-
NaCl resistance	Yeast extract-malt extract						
2%	agar with NaCl	+	+	+	+	+	+
5%		+	+	+/-	+/-	+	+/-
7%		+	+/-	-	-	+	-
10%		-	-	-	-	-	-
Temperature range	Starch casein nitrate agar	20-37℃	20-40℃	20-37℃	20-35℃	20-37℃	20-37℃

Positive = (+), negative = (-), doubtful = (+/-)

Table 4: Utilization of sugars and acid production by different isolates

Sugar	Isolate											
	2A		R3YS		N23		A26		A27		A13	
	U[a]	*A*[b]	*U*	*A*	*U*	*A*	*U*	*A*	*U*	*A*	*U*	*A*
D-glucose	+	+	+	+	+	+	+	+/-	+	+	+	+
Sucrose	-	-	+	-	+/-	-	+	-	-	-	-	-
Xylose	-	-	+	-	+/-	-	+/-	-	+	-	+	-
Inositol	-	-	+	-	-	-	-	-	+/-	-	+	+
D-Mannitol	-	-	+	+	+	+	-	-	+	+	+	+
D-fructose	+	+	+/-	+	+	+/-	-	-	+	+	+	+/-
L-Rhaminose	-	-	+	+	+	+/-	-	-	+	-	+	+
D-Raffinose	-	-	+	-	+/-	-	+/-	-	-	-	-	-
Cellulose	-	-	+	-	+/-	-	+/-	-	-	-	-	-

[a]*U = utilization of sugar as sole carbon source,* [b]*A = acid production from sugar Positive = (+), negative = (-), doubtful = (+/-)*

are the main source of clinically important antibiotics, most of which are too complex to be synthesized by combinatorial chemistry; thus, microbial natural products still appear as the most promising sources for developing future antibiotics.

In the present investigation actinomycetes were isolated from soil samples collected from different unscreened ecosystems of Punjab and Himachal Pradesh (India) which are large and diverse, for the isolation of potent and broad-spectrum antibiotic-producing actinomycetes. Out of the total 134 isolates, 51 isolates showed good activity in primary screening but failed to manifest activity in secondary screening, which is in line with results reported earlier by some researchers [16,17]. On the basis of primary and secondary screenings, six potent antibiotic producers exhibiting broad-spectrum activity were selected and studied in detail. All the six isolates grew well on most of the media tested. When grown on starch casein nitrate broth, the maximal antibiotic production was displayed on the 5th day. A broad-spectrum antifungal compound producing *Streptomyces isolate*, 1DA-28, from Indian soil, which was characterized and identified as *Streptomyces aburaviensis* var. *ablastmyceticus* (MTCC 2469) has previously been found also to exhibit maximum antibiotic production on the 5th day of incubation at 30 ºC [18].

Isolation of an antibiotic from culture filtrate is largely determined by its chemical nature. Solvent extraction is usually employed for the extraction of antibiotics from the culture filtrates. Organic solvents with different polarities have been used by many researchers for the extraction of antimicrobial compounds from actinomycetes [19-20]. Butanol was found to be the most suitable solvent for extraction of antimicrobial compounds from the culture broths of the actinomycetes studied in the present work. On the basis of cultural and morphological properties, all six isolates were classified in the genus *Streptomyces*. Previous works on novel antibiotics reported that a high proportion of organisms possessing antimicrobial activity belong to the genus *Streptomyces* [1-2]. The antimicrobial compound derived from these potent *Streptomyces* strains will be useful in developing antibiotics against drug-resistant bacteria.

CONCLUSION

The present findings highlight the significance of the six isolated *Streptomyces* strains as potential sources of potent broad spectrum antimicrobial agents. Purification and structural analysis of the active compounds from these strains may prove to be novel. Further studies along this line are underway.

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