

Streptomyces Sps - A promising source of antimicrobial agent

Abstract

The discovery of naturally occurring *actinomycetes* has a great potential in the development of the pharmaceutical industry. The soil samples were collected in the coastal area of Andaman and Nicobar. The sample was placed on Starch casein nitrate agar for 7 days at 28°C temperature. Forty three actinomycete strains were isolated. These isolates were subjected to cross streak method. The putative isolates were subjected to fermentation, and checked *in vitro* antibacterial, and antifungal activity. In conclusion, the data obtained in the present work reports that the novel metabolite from naturally occurring *Streptomyces Sp.* Was a promising source of antimicrobial agents. The characterization of the metabolite enables the discovery of new antibiotics and hence merit future studies.

Volume 7 Issue 3 - 2018

R Usha, Angima Bichang'A Kingsley

Department of Microbiology, Karpagam Academy Of Higher Education, India

Correspondence: R Usha, Department of Microbiology, Karpagam Academy Of Higher Education, Coimbatore, Tamil Nadu – 641021, India, Tel 9865068286, Email usha.anbu09@gmail.com

Received: May 15, 2018 | **Published:** June 11, 2018

Introduction

Natural products have played an important role throughout the world in treating and preventing human diseases. Secondary metabolites have been isolated and identified from marine organisms and, consequently, a compound based on the new chemical template has been developed and launched in 2004, while numerous other candidates are in clinical trials¹ marine is a natural reservoir for microorganisms and their antimicrobial products. Filamentous soil bacteria *actinomycetes* are widely recognized as industrially important microorganisms because of their ability to produce many kinds of novel secondary metabolites including antibiotics.² Secondary metabolites from *actinomycetes* have antibiotic properties it has been hypothesized that they function as a defensive measure to prevent the proliferation of competing bacteria. Competing organisms are those which consume factors, such as space and nutrients, which limit the growth and health of other organisms. Any inhibition ability from the production of an antibiotic substance gives the actinomycete a competitive advantage, thereby favoring the transmission of its genes to future generations.³

Bacteria belonging to the family Actinomycetaceae are well known for their ability to produce secondary metabolite many of which are active against pathogenic microorganisms.⁴ The *Actinomycetes* are Gram positive bacteria having high G+C (>55%) content in their DNA. The name '*Actinomycetes*' was derived from Greek 'aktis' (a ray) and 'mykes' (fungus) and given to these organisms form initial observation of their morphology.

The potential actinomycete as valuable sources for antibiotic discovery relies on the effect of growth conditions on the production of microbial secondary metabolites. A common strategy in industrial screening programs consists of growing each strain under multiple growth conditions. However, using a high number may set limits to the number of strains screened, another parameter that is critical to maximize.⁵

The genus *Streptomyces* aerobic, spore-forming *Actinomycetes*. Members of *Streptomyces* are a rich source of bioactive compounds, notably antibiotics, enzymes, enzyme inhibitors and

pharmacologically active agents (5-10). About 75 % of the known commercially and medically useful antibiotics are produced by *Streptomyces*.⁶ The natural substrates that are ideal sources for the isolation of *Actinomycetes*. Among these, they are quite commonly found in soil, water and other environments. In 1900 Beijerinck established that *Actinomycetes* occur in great abundance in the soil.

Materials and methods

Sample collection

Soil samples were collected from Andaman and Nicobar Island for the isolation of *actinomycetes* strain. These habitats include the coastal region in which the soil sample was taken from the surface soil, subsurface soil, clay loam soil, sandy clay loam and soil with fine organic material. Samples were collected and placed inside the labeled polyethylene bag. The bags were tightly closed and stored at 4°C in the refrigerator.⁷

Pretreatment of the soil sample

Each soil sample was air dried, mixed to ensure uniformity, and passed through a 2-mm pore size sieve. Enrichment of soil samples for better *actinomycetes* isolation was made according to the procedure described by Hongjuan Zhao.⁸ Three replicates of 1g of each soil sample were air-dried, sieved and then placed inside small crucibles. Those samples were subjected to drying and heat treatment inside an oven at 45°C for 12 hrs. Sieving decreased moisture retention about 1 part in 10 at tensions greater than 1.0 atm. Core samples should be used at tensions less than 1.0atm. To avoid relative errors greater than 10% (up to 30%).⁹ Enriched soils inside crucibles were incubated inside water bath at 27°C for 7 days prior to isolation.¹⁰

Isolation of actinomycetes from soil sample

Actinomycetes from the soil had been isolated by spread plate technique on Starch-casein agar after serial dilution in distilled water.¹¹ One gram from each enriched soil sample prepared as described above was suspended in 99ml of sterilized distilled water and incubated inside an orbital shaker incubator at 28°C with shaking at 140rpm for 30 min. The soil suspensions were allowed to settle at

room temperature and then used to prepare a series of further dilutions up to 10^{-4} . Aliquots of 0.1ml of each dilution were spread on the surface of Starch-Casein Nitrate Agar (SCNA) media using sterilized L-shaped glass rod. The plates were incubated for 5 days at 30°C .¹⁰ The actinomycete colony macroscopically different from each other were selected isolated and further purified by a streak plate technique. The isolated actinomycete strains were then screened with regard to their potential to generate bioactive compounds.¹²

Target organisms

The following test organisms were used for the bioassay of the antibiotic during the screening experiment *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus faecalis*, *Proteus vulgaris*, *Rhizopus* Sp. *Aspergillus niger*, *Penicillium notatum*, *Mucor* Sp. was used as a target organism in all other experiments.

Screening of actinomycetes for antimicrobial activity

Screenings of pure isolates were determined by perpendicular streak method on Muller Hinton agar (MHA). *In vitro* screening of isolates for antagonism: MHA plates were prepared and inoculated with actinomycete isolate by a single streak of inoculum in the center of the petridish. After 4 days of incubation at 28°C ¹³ the plates were seeded with test organisms (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus faecalis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*)¹¹ by a single streak at a 90° angle to Actinomycete strains. The microbial interactions were analyzed by the determination of the size of the inhibition zone.¹⁴ The isolate found to be active against all the above test organisms were further subjected to fermentation process.

Batch fermentation

The inoculum was prepared by suspending spores from a 1-week old (SCN) culture slant in 5ml of sterile saline. 1ml of the homogenous suspension containing 104-105 spores was used to inoculate 40ml of sterile Starch casein nitrate in a 250ml Erlenmeyer flask and the culture was incubated at $37\pm 2^{\circ}\text{C}$ for 5 days on a rotary shaker. Cells (grown in form of pellets) were harvested in a sterile centrifuge tube (25ml) by centrifugation at 10,000 rpm for 10 min.¹²

In vitro antibacterial assay

The antibacterial activity of crude metabolite was tested by Disk diffusion assay.¹⁵ The plates were incubated at 37°C for 24h during which activity was evidenced by the presence of a zone of inhibition surrounding the well. Replicated were done and the antibacterial activity was expressed as the mean of diameter of the inhibition zones (mm) produced by the secondary metabolite when compared to the controls.

In vitro antifungal assay

Antifungal activity of the crude metabolite was determined by using the standard method.¹⁵ The fungal cultures were maintained in 0.2% dextrose medium and the optical density 0.10 at 530nm was adjusted using spectrophotometer. Each fungal inoculum was spread on Sabouraud's Dextrose agar using a sterile swab. Disk diffusion assay was followed to evaluate the antifungal activity. The Petri plates were incubated at 30°C for 2 days. At the end of the 48 hrs, inhibition zone was formed in the medium were measured in millimeters (mm). Replicated were done and the antifungal activity was expressed as

the mean of diameter of the inhibition zones (mm) produced by the secondary metabolite when compared to the controls.

Results and discussion

Isolation of actinomycetes

Of the 5 soil sample, a total of different 43 *actinomycetes* strains were identified by using the Starch casein nitrate agar medium were showed in (Table 1). This medium seems to be specific and sensitive for *actinomycetes* (Figure 1). The *actinomycetes* colony macroscopically different from other selected isolates were further purified by streak plate technique. The pure culture was further maintained at 4°C for further use.

Patil et al.¹⁶ were enumerated and expressed actinomycetes isolates as CFU per gram of sediment. Nurettin et al.¹⁷ recovered 74 different *actinomycetes* from 10 soil samples. Similarly (Mustafa et al.¹⁸) reported fifty different *actinomycetes* from farming soil sample. Occurrence of *actinomycetes* in the sediment of marine and estuarine environments.¹³ The population density of *actinomycetes* in the soil might be determined by the soil nutrients like total organic carbon, nitrogen, and phosphorous reported by.¹⁹ Most of the isolates tend to grow in soils, which is an important characteristic feature of *actinomycetes*¹⁹ and with adequate source of carbon and nitrogen present. Colonies of *actinomycetes* were recognized by their characteristic chalky to leathery appearance.

Selection of isolates producing antibacterial substances

Antibacterial activities of isolates were tested preliminarily by cross streak method. Potential antagonistic *Actinomycetes* strain was identified by using perpendicular streak method on muller hinton agar plate. Growth inhibition near to the centrally streaked soil isolates was an indication of the production of antibacterial substances. The results determined that from 43 isolates, 15 *actinomycetes* isolates (KUA 103, KUA 104, KUA 106, KUA109, KUA 110, KUA 111, KUA 113, KUA 114, KUA 122, KUA 122, KUA 136, KUA 137, KUA 139, KUA 157, KUA 160, KUA 161) showed antagonistic activity against all the tested pathogens (*Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus faecalis*) and Gram negative (*Pseudomonas aeruginosa*, *Proteus vulgaris*, *Escherichia coli*). Of 15 isolates, 6 strains (KUA 103, KUA 104, KUA 106, KUA 122, KUA 137, KUA 139) showed remarkable results towards antibacterial activity and these 6 strains were taken for our study (Table 2). The microbial interaction was analysed by the size of the inhibition zone were showed in (Figure 2)

These results compare with other investigations Patil et al.¹⁶ reported that the cross streak method was used to detect the inhibitory strains among the isolates of *actinomycetes*. *Actinomycetes* strains were streaked across the diameter on to yeast extract glucose agar plates with a width of the break being 8-10mm. After incubation at room temperature for a period of 5-7 days, young culture of each test organisms (such as *Aeromonas hydrophila*, *A. sobria*, and *Edwardisella tarda*) was streaked horizontally to the central strip of the *actinomycetes* culture, 1-2mm apart from the central streak. The plates were re-incubated at room temperature for 24 hrs. The inhibitory activity of the *actinomycetes* isolates was indicated by absence of growth near the central strip.

Mohan remya et al.²⁰ determined that out of 64 strains, 21 isolates (32.8%) had antimicrobial activity, of which 12 isolates (18.8%) showed antibacterial activity, 13 isolates (20.3%) showed antifungal

activity (against *C. albicans*); 9 isolates (14.1%) showed both antibacterial and antifungal activity.

Ozgun et al.² screened fifteen *streptomyces* isolates which exhibited antimicrobial activity against at least two of the test strain *S.aureus* strains including methicillin resistant *Staphylococcus aureus* (MRSA) Twelve *Streptomyces* isolates showed, inhibition on gram negative bacteria tested. Similarly Penka et al.²¹ showed antibiotic activity of selected strains using the test pathogens. It was found that 18 strains suppressed in different degree of the test microorganisms.

Selection of active actinomycete strains

Of the 43 isolated strains, 6 *actinomycetes* strain showed greater activity against gram negative and gram positive bacteria. It could be suggested that the produced antibiotic substances had a broad spectrum of activity. For this reason we focused on the strains, which proved to possess such activity, selected strains were KUA106, KUA103, KUA104, KUA122, KUA137, and KUA139.

Batch fermentation

The fermentation process was carried out for 6 days at 30°C using starch casein nitrate broth as production medium. Growth appeared in the form of pellets. Production of active compound was slow and it was extracellular. The production started after 24 hrs, gradually reached maximum at 72 hrs, and maintained at the same level upto 120 hrs. Simultaneously, an increase in pH to 8.13 was noticed after 120 hrs. Biomass gradually increased and reached maximum at the same level up to 72 hrs and maintained at the same level up to 144 hrs, indicating no autolysis. The total volume filtered was conducted followed by centrifugation at 5,000 rpm at 10°C for 20 minutes.

Atta et al.¹² who worked on *Streptomyces violaceuniger* reported that they inoculated in to 250ml Erlenmeyer flasks containing 75ml of liquid starch nitrate medium kept on 200 rpm at 30°C for 5 days. Similarly results were reported by Shatoury et al.²² preserved spore suspension of *actinomycetes* in to 30ml of starch casein nitrate medium on 200 rpm for 5 to 7 days at 28°C. Jin et al.²³ ferment *Streptomyces scabiei* M0137 on oat meal broth at 30°C for 7 days on a rotary shaker at 180 rpm. The 7-day cultured broth was centrifuged at 10,000rpm for 20 minutes.

In vitro antibacterial activity of culture filtrate

After screening by cross streak method, 6 strains were selected for the anti bacterial activity using bacterial pathogen. The isolates showed antibacterial activity against gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus faecalis*) and Gram negative (*Pseudomonas aeruginosa*, *Proteus vulgaricus*, *Escherichia coli*). The formation of inhibition zone around the pathogenic strain was due to the production of secondary metabolite (Figure 3). Results of selected *actinomycetes* culture filtrate, KUA 104 and KUA 139 showed activity towards both gram positive and gram negative organisms. KUA 122 showed activity towards gram negative organisms. KUA 103, KUA 137 showed activity towards gram positive organism (Table 3). It was found that 6 strains showed antagonistic activity in different inhibition rate. Of the 6 isolates mentioned earlier KUA 106, showed highest inhibition zone (15mm) on *Proteus vulgaricus*, (10mm) on *Bacillus subtilis* and *Streptococcus faecalis*, (13mm) on *Pseudomonas aeruginosa*, (12mm) on *Escherichia coli* and (8mm) on *Staphylococcus aureus* showed a remarkable highest activity towards both gram positive and negative microorganisms. It is interesting to note that this response of KUA 106 represents an antibiotic potential competing microorganism against *proteus*

vulgaricus. The selected six strains possessed antibacterial activity against the pathogens (Figure 4).

Suthindhiran et al.¹⁵ showed antagonistic activity against gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* using the *actinomycetes* species *Saccharopolyspora Salina*. Antibacterial activity of *Streptomyces* against *Escherichia coli*, *Shigella boydi*, *Pseudomonas aeruginosa*, *Streptococcus Sp.*, *Staphylococcus aureus* and *Klebsiella pneumonia* was showed by Shatoury et al.²².

Similarly Ozgun et al.² used six bacteria, including three gram positive (*Staphylococcus aureus* MU 38, *Staphylococcus aureus* MU 40, *Staphylococcus aureus* ATCC 25923) and three Gram negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Stenotrophomonas maltophilia* MU 64) and one yeast (*Candida albicans* ATCC1023) to determine antimicrobial activity of the isolated *Streptomyces* strains

In vitro antifungal activity of culture filtrate

Among the 43 isolates of *actinomycetes*, six isolate were selected for antifungal activity. The assay carried out using the fermented broth utilized by the isolates. *Penicillium crysogenum*, *Aspergillus niger*, *Aspergillus flavis*, *Mucor Sp.*, *Rhizopus Sp.* and *Fusarium Sp.* was the fungal strains used for the antifungal activity. Results of KUA 104, KUA 106 showed antifungal activity against all the pathogenic strain. KUA 103 showed activity against test pathogen except *Fusarium Sp.*, KUA 139 showed antifungal activity against the pathogens except *Aspergillus niger*, and KUA 122 showed antifungal activity against the pathogens except *Penicillium crysogenum* (Table 4). Of the selected isolates KUA 106 showed broad spectrum activity (11mm) on *Penicillium crysogenum*, (8mm) on *Aspergillus niger* and *Aspergillus flavis*, (4mm) on *Mucor Sp.*, (10mm) on *Fusarium Sp* and (6mm) on *Rhizopus Sp* (Figure 5). As *Aspergillus niger* was such a common contaminant, highly effective antibiotics are required, among the tested isolates KUA 106 showed strong antifungal activity.

Asha et al.²⁴ found *actinomycetes* showed antifungal activity against *Aspergillus niger* but not against *Candida albicans*. Anka et al.²⁵ determined antagonistic spectra of the 28 strains of *actinomycetes* against 9 spp of storage fungi and 4 species of field fungi.

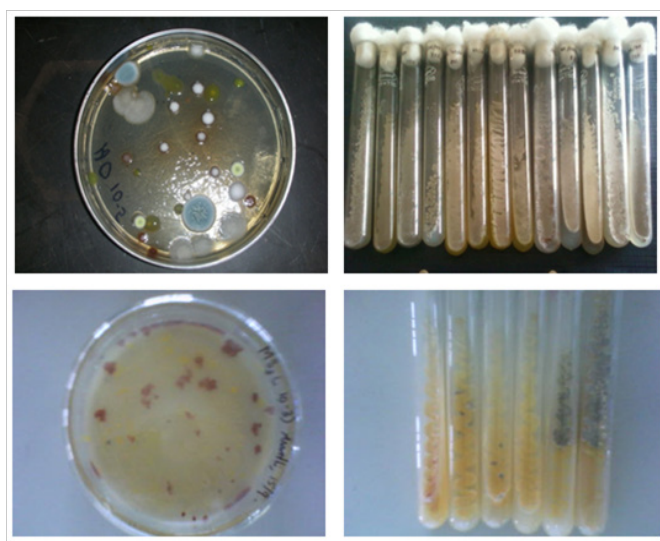


Figure 1 Isolated colonies on starch casein nitrate agar.

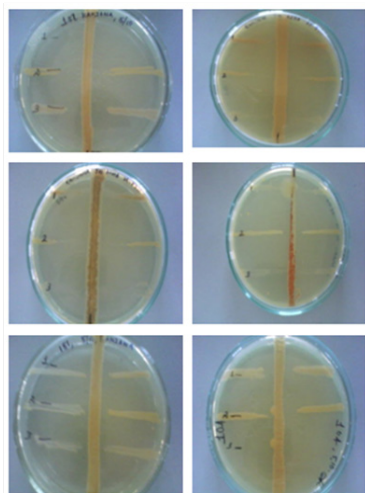


Figure 2 Antagonistic activity of actinomycetes by cross streak method.

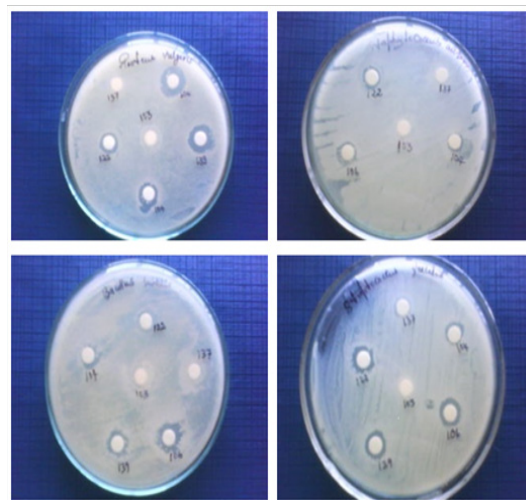


Figure 3 Antibacterial activity of KUA 106 strain.

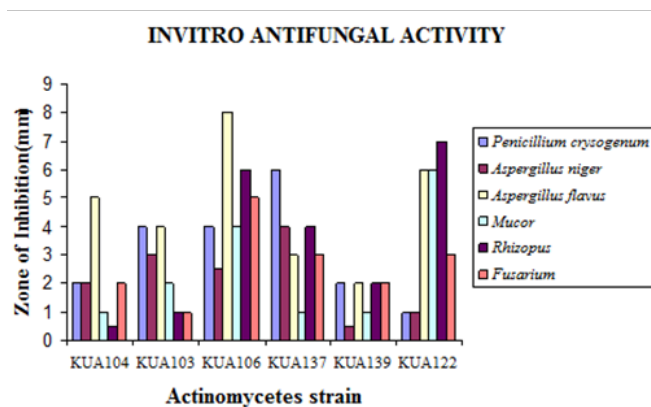


Figure 4 In vitro antibacterial activity.

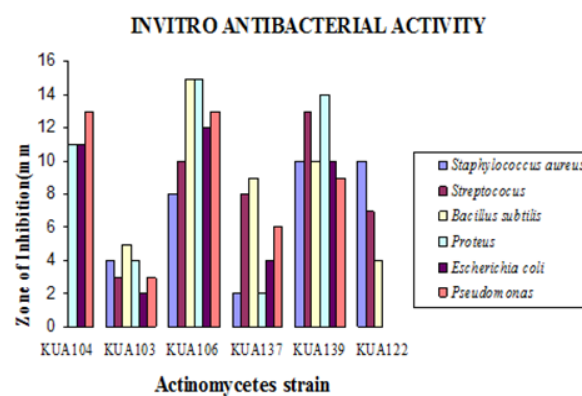


Figure 5 In vitro antifungal activity.

Table 1 Isolation of actinomycetes on starch casein nutrient agar

Origin of soil sample	Total number of isolate(CFU)(unit/ml)
Surface soil (0-15cm)	10×10 ⁶
Subsurface soil (15–30cm)	15×10 ⁶
Clay loam in nature, with high organic carbon content (1.5%).	7×10 ⁶
Sandy clay loam with medium organic carbon content (0.55%)	20×10 ⁶
Soil with fine organic material	15×10 ⁶

Table 2 Antibacterial activity of isolated Actinomycetes strains against pathogenic bacteria

Actinomycetes strain	Test bacteria ,Inhibition zone (mm)					
	1	2	3	4	5	6
KUA 101	-	-	22	-	20	14
KUA102	-	-	-	-	1	-
KUA103	12	16	14	22	33	18
KUA104	18	18	24	34	17	21
KUA105	-	-	-	-	1	2
KUA106	30	29	25	30	31	30
KUA109	10	25	18	19	20	13

Table Continued

KUA110	16	21	10	3	13	18
KUA111	22	-	39	13	-	-
KUA112	-	-	5	-	-	1
KUA113	10	13	17	21	15	10
KUA114	14	13	15	10	18	10
KUA115	-	-	2	-	-	1
KUA120	-	31	10	18	13	15
KUA122	15	20	24	28	25	27
KUA124	-	-	-	-	-	-
KUA125	22	19	-	-	-	-
KUA126	-	-	11	-	10	19
KUA127	-	-	-	-	11	-
KUA129	-	-	12	-	-	28
KUA131	-	-	-	-	-	-
KUA132	-	33	-	-	31	15
KUA133	-	-	-	-	-	-
KUA134	-	25	33	10	10	-
KUA136	22	14	16	33	22	22
KUA137	30	20	24	25	30	30
KUA139	28	18	28	13	22	30
KUA140	-	30	34	13	-	22
KUA141	-	15	11	-	-	10
KUA143	-	14	25	13	22	-
KUA144	1	-	-	-	-	17
KUA146	-	26	26	15	-	12
KUA148	-	-	19	-	-	14
KUA150	-	14	13	23	27	-
KUA151	-	-	-	-	-	-
KUA152	-	-	-	-	-	-
KUA155	-	-	10	13	20	-
KUA157	14	15	31	14	17	17
KUA159	-	-	-	-	-	-
KUA160	22	12	44	22	31	2
KUA161	11	41	4	2	33	22
KUA162	-	-	25	-	-	-
KUA163	-	-	-	-	-	-
KUA164	-	33	24	25	-	16

1: *Staphylococcus aureus*, 2: *Streptococcus faecalis*, 3: *Bacillus subtilis*, 4: *Proteus vulgaris*, 5: *Escherichia coli*, 6: *Pseudomonas aeruginosa*Table 3 *In vitro* antibacterial activity of culture filtrate

Test bacteria	Actinomycetes strain Zone of inhibition (mm)					
	KUA104	KUA103	KUA106	KUA137	KUA139	KUA122
<i>Staphylococcus aureus</i>	9	4	8	-	10	10
<i>Streptococcus faecalis</i>	11	-	10	8	13	7
<i>Bacillus subtilis</i>	10	-	10	15	10	-
<i>Proteus vulgaris</i>	11	-	15	-	14	10
<i>Escherichia coli</i>	11	-	12	-	10	13

Table Continued

<i>Pseudomonas aeruginosa</i>	13	-	13	6	9	13
-----------------------------------	----	---	----	---	---	----

Table 4 *In vitro* antifungal activity of the culture filtrate

Test fungi	Actinomycetes strain Zone of inhibition (mm)					
	KUA104	KUA103	KUA106	KUA137	KUA139	KUA122
<i>Penicillium crysogenum</i>	2	4	11	6	2	-
<i>Aspergillus niger</i>	8	3	8	4	-	1
<i>Aspergillus flavus</i>	5	7	8	3	5	6
<i>Mucor</i>	1	2	4	-	8	6
<i>Rhizopus</i>	6	6	6	4	2	7
<i>Fusarium</i>	2	-	10	3	2	3

Conclusion

Today, metabolite, as naturally produced antimicrobial proteins, have rather interesting potential of application in pharmaceutical industry and act as a factor in humans' Therefore, new protection principles are developing, including the application of *actinomycetes* metabolite in preventing the survival of this microorganism. Metabolite production by *Actinomycetes* enables selective and competitive effect on pathogenic microorganisms.

Acknowledgements

None.

Conflict of interest

The author declares that there is no conflict of interest.

References

- Orooba MF, Ali AS, Khansa MY, et al. Isolation, screening and antibiotic profiling of marine *Actinomycetes* extracts from the coastal of Peninsular Malaysia. *Int J ChemTech Res.* 2017;3:212–224.
- Kekuda PTR, Onkarappa R, Jayanna ND. Characterization and antibacterial activity of a glycoside antibiotic from *Streptomyces variabilis* PO-178. *Sci Technol Arts Res.* 2014;3:116–121.
- Marelle L. The Search for Novel Secondary Metabolites from Marine Obligate *Actinomycetes*. 2005.
- Harald B, Espen F, Geir J, et al. *Actinomycetes* from Sediments in the Trondheim Fjord, Norway: Diversity and Biological Activity. *Mar Drugs* 2008;6(1):12–24.
- Seizo S, Iwata F, Mukai T, et al. Indoxamycins A–F. Cytotoxic Tricyclic Polypropionated from a Marine-Derived *Actinomycete*. *The Journal of Organic chemistry.* 2009;63(12):1682–1683.
- Sahar A, Shatoury EL, Nahla S, et al. Antimicrobial, antitumor and *in vivo* cytotoxicity of *actinomycetes* inhabiting marine shellfish. *World J Microbiol Biotechnol.* 2009;25(9):1547–1555.
- Hesham M. Enhancement of Rice Straw Composting by Lignocellulolytic *Actinomycete* Strains. *Int J of agriculture and boil.* 2007;1:106–109.
- Hongjuan Z, Rachel L, Parry, et al. The rapid differentiation of *Streptomyces* isolates using Fourier transform infrared spectroscopy. *Vibrational Spectroscopy.* 2006;40(2):213–218.
- Singh V, Haque S, Singh H, et al. Isolation, Screening, and Identification of Novel Isolates of *Actinomycetes* from India for Antimicrobial Applications. *Front Microbiol.* 2016;7:19–21.
- Taechowisan T, Lumyong S. Activity of endophytic *actinomycetes* from roots of *Zingiber officinale* and *Alpinia galanga* against *phytopathogenic fungi*. *Annals of Microbiol.* 2003;53(3):291–298.
- Juby TR, Usha R. Antibacterial finish for cotton fabric from *actinomycetes* isolated from forest soil. *JPAM.* 2018;12(1):333–341.
- Atta HM, SM Dabour, Desoukey SG. Sparsomycin Antibiotic Production by *Streptomyces* Sp. AZ–NIOFD1: Taxonomy, Fermentation, Purification and Biological Activities. *American–Eurasian J Agric & Environ Sci.* 2009;5 (3):368–377.
- Kathiresan K. How do mangrove forests induce sedimentation? *Revista de Biologia Tropical.* 2003;51(2):355–360.
- Mohammad RA, Ghiasian SA. Isolation and Characterization of Medically Important Aerobic *Actinomycetes* in Soil of Iran. (2006 – 2007). *Open Microbiol J.* 2009;3:53–57.
- Suthindhiran K, Kannabiran K. Cytotoxic and Antimicrobial Potential of *Actinomycete* Species *Saccharopolyspora salina* VITSDK4 Isolated from the Bay of Bengal Coast of India. *American J of Inf Diseases.* 2009;5(2):90–98.
- Patil R, Jeyasakaran G, Shanmugam SA, et al. Control of bacterial Pathogen, associated with fish diseases, by antagonistic marine *Actinomycetes* isolated from marine sediments. *Indian j of mari sci.* 2001;30(4):264–267.
- Nurettin S. Investigation of the Antimicrobial Activity of Some *Streptomyces* isolates. *Turk J Biol.* 2002;27:79–84.
- Mustafa O. Comparison of *Streptomyces* diversity between agricultural and non-agricultural soils by using various culture media. *Scientific Research and Essay.* 2009;4(10):997–1005.
- Thangapandian V, Ponmurugan P, Ponmurugan K. *Actinomycete* diversity in the rhizosphere soil of different medical plants in kolly hills –tamil nadu , India, for the metabolite production. *Asian j of plant sci.* 2007;6(1):66–70.
- Mohan R, Ramya V. Isolation and characterization of marine Antagonistic *Actinomycetes* from west coast of India. *Series: Medicine and Biol.* 2008;15(1):13.
- Penka M, Sava T, Dimitrova N, Chipeva V, Nikolova SA, Nevena B. Characteristics of soil *Actinomycetes* from antarctica. *J of culture collection.* 2002;3:3–14.
- El-Shatoury SA, El-Shenawy NS, Abd El-Salam IM. Antimicrobial, antitumor and *in vivo* cytotoxicity of *actinomycetes* inhabiting marine shellfish. *World J Microbiol Biotech.* 2009;25:1547–1555.
- Jin Y, Kim JH, Jung W, et al. Production and Biological Activity of Laidlomycin, Anti-MRSA/VRE Antibiotic from *Streptomyces* sp. CS684. *J Microbiol.* 2007;45(1):6–10.
- Asha devi NK, Jeyarani M, Balakrishnan K. Isolation and identification of *Actinomycetes* and their potential in antimicrobial activity. *J of biol sci.* 2006;9(3):470–472.
- Anka R, EG Welty, B Lucas. Antifungal Spectra of *Actinomycetes* Isolated from Tobacco. *Antimicrob Agents Chemother.* 1972;29:363–365.