Research article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

Actinobacteria *Sterptomyces* sp. as potent inhibiting agent against Phytopathogens

G. Vaijayanthi*, A. Savithri and P. Gajalakshmi

Research Department of Microbiology. Dhanalakshmi Srinivasan College of Arts and Science for Women (Autonomous), Perambalur-621212
Email: gvaijayanthimicro@gmail.com

ABSTRACT

In this present study was performed to isolate soil actinobacteria colonies having antifungal activity from soil sample. Totally 15 actinobacteria were isolated from collected soil sample using Starch casein agar medium by streak plate method. All the isolates were screened for their antifungal activity by well diffusion method against five phytopathogenic fungi. Of this, 7 isolated strains inhibits all the pathogens, 3 strains inhibit both *Alter aria* sp. and *Fusarium oxysporum*, 3 strains inhibits *Aspergillus niger*, 2 strains inhibit *Penicillium* sp. and *Rhizoctonia solani*. The metabolites from potent strain GV7 was produced by fermentation, separated by centrifugation, it was tested for their antifungal activity against the test fungal strains by well diffusion method. In this study, the GV7 (identified as Streptomyces sp.) have showed good antifungal activity. While all the isolates showed inhibitory activity against Phyto-pathogenic fungi, it is suggestive that terrestrial soil could be an interesting source to determine for antifungal secondary metabolites.

KEYWORDS: Soil sample, Actinobacteria, Phytopathogen, Antifungal activity

***Corresponding author**

Dr. G. Vaijayanthi

Department of Microbiology

Dhanalakshmi Srinivasan

College of Arts and Science for Women (Autonomous)

Perambalur-621212

Email: gvaijayanthimicro@gmail.com

INTRODUCTION

Fungal phytopathogens cause serious problems worldwide in agriculture and food industry by destroying crops and economically important plants. Fungicidal or synthetic compounds which are used to keep fungal infections at an acceptable level, are associated with several drawbacks such as their lack of specificity, accumulation if biodegradation is slow or even missing, and others are toxic not only to fungi but also to other beneficial life forms and also have led to environmental pollution and development of pathogen resistance. Due to worsening problems in fungal disease control, alternate methods for plant protection are needed which are less dependent on chemicals and more environmentally friendly which is potential use of biocontrol agents as replacements or supplements for agrochemicals.[8, 15]

Soil Actinobacteria have revealed their wide antifungal activity[10]. They have been shown to protect several plants to various degree of soil borne fungal pathogens. Actinobacteria as biocontrol agent, produce Urauchimycins which is a member of antimycin class, a set of well-identified antifungals, that act by inhibiting the electron flow in the mitochondrial respiratory chain of a phytopathogenic fungus and have been identified in Streptomyces isolated from the integument of attendants[6]. Around 23 000 bioactive secondary metabolites by microorganisms have been reported and over 10000 of these compounds are produced by Actinobacteria[16]. Among these around 7600 compounds are produced by *Streptomyces* species. 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. As the frequency of novel bioactive compounds discovered from terrestrial actinobacteria decreases with time, much attention has been focused on screening of actinobacetria from diverse environments[11]. They have been looked upon as a potential source of antibiotic and the past experience proves that actinobacteria are the richest source of secondary metabolites[1].

In the present investigation an effort was made to screen antagonistic terrestrial actinobacteria from soil of agricultural field which is largely unscreened ecosystem for the isolation of potent bioactive compound producing terrestrial actinobacteria

MATERIALS AND METHODS

Sample collection and processing:

Soil sample were collected from agricultural field in Tiruchirappalli District, Tamilnadu India. The collected samples were stored into a sterile glass screw cap bottle and scaled tightly. The soil samples were transferred to plastic tray for air drying in shadow condition. After drying, the sample were weighed and crushed with a pestle and mortar and sieved the total sample through a 2 mm sieve. Then the crushed soil sample were labelled and stored in plastic boxes in a refrigerator for

further use.

Isolation of actinobacteria

Starch casein agar (SCA) as recommended by kuster and Williams medium for the isolation of actinobacteria was prepared and sterilized at 121^0C in 15 Ibs pressure for 15 min. The medium was poured into sterile petri dishes and were allowed to solidity. The collected soil samples were diluted up 10^{-6}, and 0.1 ml of the diluted samples was spread over the agar plates. The plates were incubated at 28±2°c for seven to ten days. After incubation, the actinobacterial colonies were observed, purified using subculture method and maintained in SCA medium for further assay.

Production of bioactive compounds

Based on the preliminary screening, the antagonistic natures of actinobacteria were conformed and it was further studied by shake flask fermentation technique. The potential strains were inoculated in 50ml of freshly prepared sterile yeast extract malt extract broth medium (ISP2) in a 250ml conical flask. The flasks were placed on shaker at 120rpm for 7-8 days. After incubation, the cells free supernatant was separated by centrifugation at 10000 rpm for 10 minutes and compound[4].

Antagonistic effect against pathogenic fungi

The actinobacterial isolates were evaluated for their antagonistic activity against five phytopathogenic fungi such as *Alternaria* sp, *Fusarium oxysporum*, *Penicillium* sp., *Rhizoctonia solani* and *Aspergillus niger* by dual-culture invitro assay. Fungal discs (8mm in diameter), 5 days old on GYE at 28ºC were placed at the center of GYE plates. Two actinobacteria discs (8mm) 5 days old, grown on starch casein agar, incubated at 28ºC were placed on opposite sides of the plates, 3 cm away from fungal disc. Plates without the actinobacteria disc serve as controls.

All the plates were incubated at 28ºC for 14 days and colony growth inhibition (%) was calculated by using the formula: C T/C x 100. Where, C is the colony growth of pathogen in control and T is the colony growth of pathogen in dual culture. The zone of inhibition was measured between the pathogen and actinomycetes isolate.

Well diffusion method

The test fungal cultures as mentioned in preliminary screening were inoculated into freshly prepared SDA plates (fungal strains) using sterile cotton swabs. Then the wells were made (about 5mm in dia) on the all inoculated plates using well cutter and each well was loaded with 100 µl of cell free culture supernatant. The plates containing fungal strains were incubated at room temperature for 4-5 days. After incubation, the zone of inhibition was measured and expressed as millimeter in diameter[3].

Characterization of actinobacteria

Colony characterization:

Colony morphology of the actinobacteria was recorded with respect to colour of aerial and substrate mycelia, size and nature of the colonies and production of diffusible pigmentation.

Microscopic characterization:

Actinobacterial culture plates were prepared and 2 to 4 sterile cover slips were inserted at an angle of 45°C. The plates were incubated at 28°C ± 2°C for 4 to 7 days. The cover slips were slowly removed from the cultured medium and observed under the light (Nikon, Japan) microscope. The morphological features of spores and sporangia on aerial and substrate mycelia was observed and recorded.

Identification of actinobacteria:

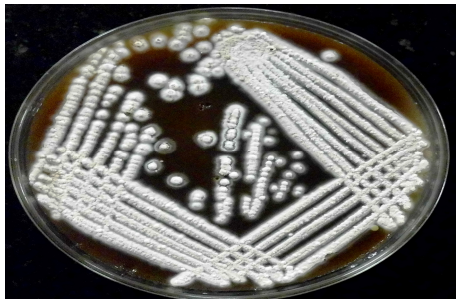
In addition to morphological characterization, biochemical properties of the actinobacteria were also performed and identified by using standard manuals[9].

High performance liquid chromatography (HPLC) chromatography

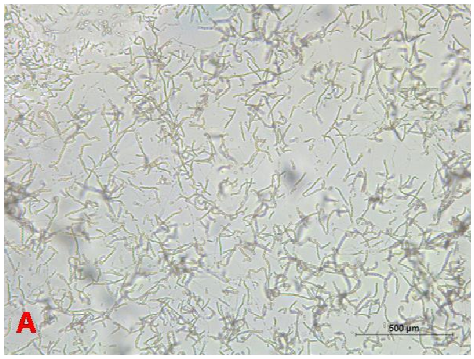
Qualitative analysis was performed by silica gel thin-layer chromatography with a solvent mixture of petroleum ether: acetone (19:1, v/v) as mobile phase and the development was observed under ultraviolet lamp. Separation of carotenoids was also carried out by HPLC on a C18, 3µm column with acetonitrile: methanol: propanol (40:50:10). The flow rate was 0.8 ml/min[9].

RESULT AND DISCUSSION

Totally 15 morphologically different actinobacterial colonies were isolated from collected soil samples by streak plate method on starch casein agar medium. All the isolated strains were screened for antifungal activity against five fungal phyto-pathogens. Of this 7 strains inhibits all the pathogens, 3 strains inhibit both *Alternaria* sp. and *Fusarium oxysporum*, 3 strains inhibits *Aspergillus niger*, 2 strains inhibit *Penicillium* sp. and *Rhizoctonia solani* (Table 1). The results revels that the active isolates GV7 inhibit all the phytopathogens followed by GV2 moderate inhibition and GV4, GV5, GV9, GV11, GV14 poor inhibition of all the pathogens.



Morphological Characterization of Potent Actinobacteria-GV7



Microscopic Characterization of Potent Actinobacteria –GV7

Table-1.Screening of actinobacteria for antagonistic activity

Strains code	*Aspergillus niger*	*Alternaria* sp.	*Fusarium oxysporum*	*Penicillium* sp.	*Rhizoctonia solani*
GV1	-	-	-	8	7
GV2	6	8	6	7	7
GV3		7	7	-	-
GV4	4	6	5	4	4
GV5	3	5	4	5	4
GV6	-	5	6	-	-
GV7	11	14	15	12	11
GV8	-	-	-	7	6
GV9	4	6	4	5	4
GV10	-	5	4	-	-
GV11	3	5	6	4	5
GV12	3	-	-	-	-
GV13	4	-	-	-	-
GV14	3	7	4	7	4
GV15	4	-	-	-	-

Zone of inhibition in 'mm'; - no inhibition

In the present study well diffusion method was used for the detection of antifungal activity. This method allowed utilizing very small amount of medium for the culturing and production of bioactive compounds and also for the detection of antifungal activity of more number of actinobacterial isolates against wide range of pathogens with less investment costs[3].

The potential actinobacteria was selected based on the results in preliminary screening of actinobacteria for antifungal activity. In that seven actinobacterial strains inhibit five pathogens, in which only one actinobacterial isolate namely GV7 which showed inhibition of all the five tested fungal pathogens, which was selected as potent strain and used for fermentation.

In well diffusion method the crude culture filtrate of actinobacterial strain (GV7) showed good activity against all the tested fungal strains (Table 2). Actinobacteria are the most biotechnologically valuable prokaryotes responsible for the production of about half of the

discovered bioactive secondary metabolites including antibiotics[17]. They are the main source of bio-control agent, 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[2].

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 actinobacteria[8]. This result clearly indicated that the antifungal activity of potential strain is due to the production of extracellular bioactive compounds. The published literature stated that most of the antibiotics from actinobacteria are extracellular in nature[13].

Antifungal Activity of Actinobacteria zone of inhibition

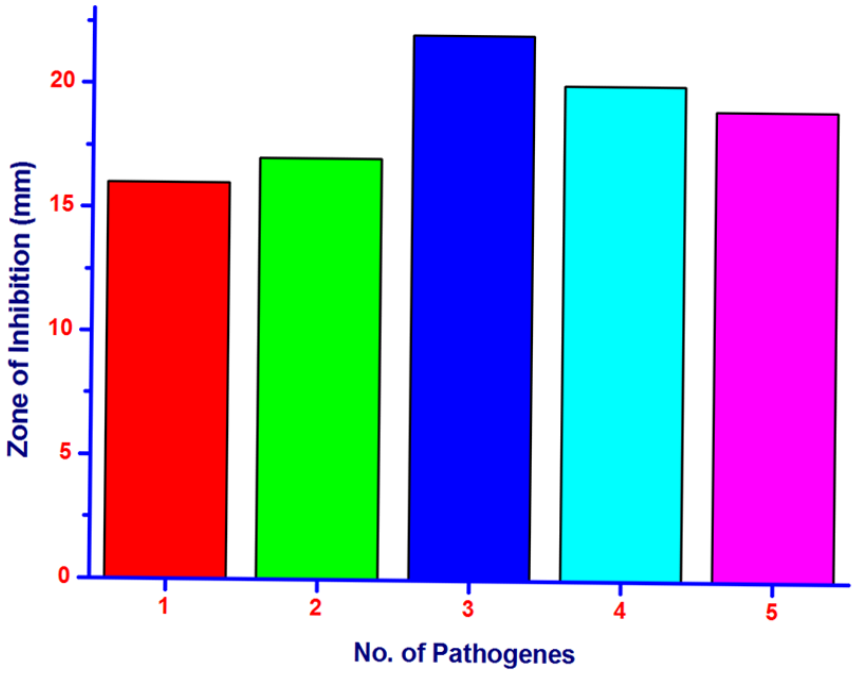


Table-2: Antifungal Activity of Actinobacteria by well diffusion method

S.No	Name of the pathogens	Zone of the inhibition (mm)
1	*Aspergillus niger*	16
2	*Alternaria* sp.	17
3	*Fusarium oxysporum*	19
4	*Penicillium* sp.	17
5	*Rhizoctonia solani*	16

Table-3 Characterization of potential isolate GV7

S.No	Name of the test	Results
1	Presence of aerial and substrate mycelium	+
2	Spores in aerial mycelium	+
3	Spores in substrate mycelium	-
4	Sporo spore morphology	Recti flexibles
5	Spore surface	Smooth
6	Shape of spores	Globes
7	Aerial mycelium colour	Ash
8	Reverse side colour	Pale yellow
9	Colony size (mm)	4
10	Gram staining	+
11	Acid fast	Non acid fast
12	Indole	+
13	Methyl red	+
14	Voges proskauer	+
15	Citrate utilization	+
16	Catalase	+
17	Urease	+
18	Nitrate	+

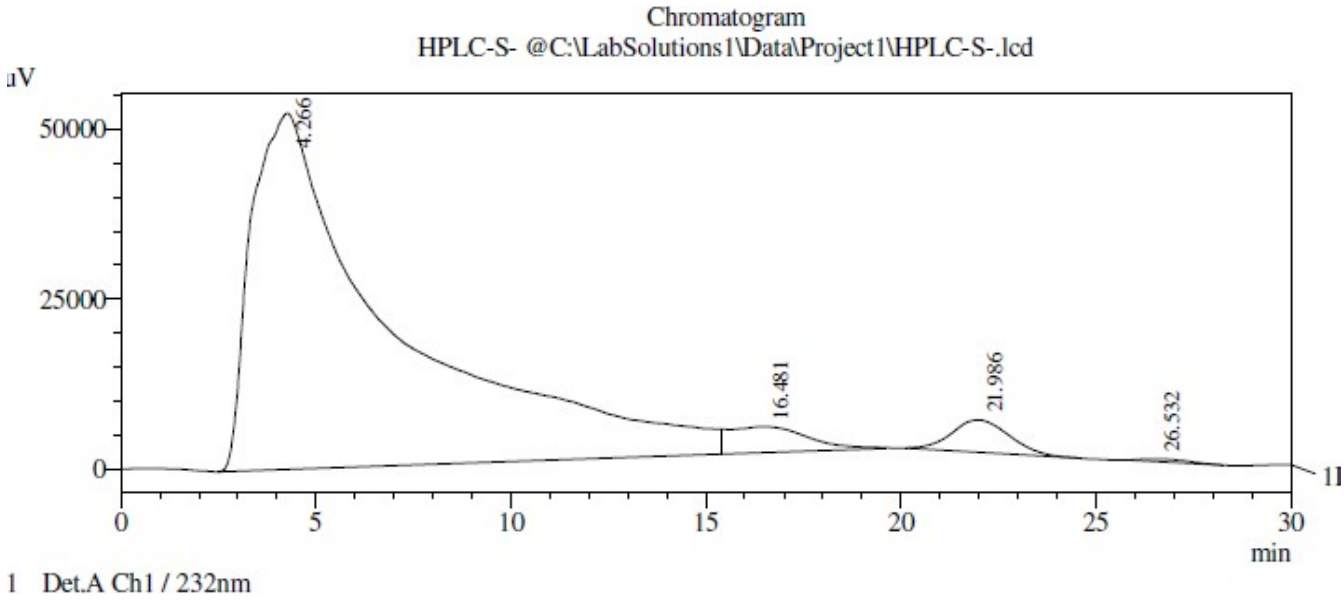
. Classical approaches for the classification make use of morphological, biochemical character, colony formation vegetative and mycelium structure of spores and spores are the most important feature of identification of actinomycetes. In the present investigation potential actinobacterial strain GV7 were identified by morphological, cultural and biochemical characteristic (Table-3).

The pale yellow, white, Brown and ash coloured isolates were found predominant such as dominance of members of pale yellow, brown and white coloured actinobacteria. Under microscopic observation, strain GV7 showed the presence of substrate and aerial mycelium with rectus flexible (RF) arrangement of spore chains.

Biochemical characteristics of the actinobacterial isolates GV7 were also used as the character of identification. In the present study biochemical characteristics such as Indole, Methyl Red, Voges Proskauer, Citrate, Urease, Nitrate and Catalase test were used to characterized the actinobacterial isolate (Table-3).

Based on the morphological, biochemical, cultural features, the potent actinobacterial isolates namely GV7 identified as *Streptomyces* sp. GV7. Actinobacterial isolates produced the ash series colour of aerial mycelium, and sixteen isolates produced white series colour of aerial mycelium on starch casein agar medium, and seven isolates produced diffusible pigments on the same medium. Thus, it is concluded on the basis of present and previous studies that the, the physical and chemical properties and nutrient composition of the media also influenced the isolation/cultivation and morphological properties of the soil actinobacteria. Similar type of findings also reported by many workers [5, 12, 14].

In the present study, extract of isolate GV7 showed two peaks in HPLC graph, first peak at retention time of 2.3 min and second peak at retention time of 3.9 min. After comparison of theses peaks with standards, it was concluded that second peak resembled with the standard peak of Rifampin, so it may be said that sample A1 is producing Rifampin with some other compounds. Rifampin is an antibiotic used against many pathogens, with purported antidepressant and aphrodisiac properties. Thus the isolates are producing commercially useful antibiotics.



CONCLUSION

Findings of the present study conclude that terrestrial environment is the potential ecosystem for antagonistic actinobacteria which deserves for bioprospecting. Considering the mentioned above results, it could be seen that one from the investigated strains (GV7) exhibited higher activity against pathogenic fungi. This actinobacteria (GV7) have a potential to be included in researches of new preparations with antifungal action also for plant protection.

REFERENCE

1. Ashadevi N.K. Isolation and identification of marine actinomycetes and their potential in antimicrobial activity. *Pakistan J. Biological Sciences.* 2005; 9(3): 470-472.
2. Deepika Sharma, Talwinder Kaur, Chadha B.S., Rajesh Kumari Manhas. Antimicrobial Activity of Actinomycetes Against Multidrug Resistant Staphylococcus aureus, *E. coli* and Various Other Pathogens. *Tropical Journal of Pharmaceutical Research.* 2011;10 (6): 801-808.

3. Mohanraj D., Bharathi S., Radhakrishnan M., Balagurunathan R. Bioprospecting of actinobacteria from Yelagiri hills with special reference to antibacterial activity. *J. Chem. Pharm. Res.* 2011; 3(3):439- 446.
4. Radhakrishnan M., Balaji S., Balagurunathan R.Thermotolerant actinomycetes from the Himalayan mountain-antagonistic potential, characterization and identification of selected strains. *Malaysian Applied Biology*. 2007; 36 (1): 59-65.
5. Raja, A., Prabakaran, P. and Gajalakshmi, P. Isolation and screening of antibiotic producing psychrophilic actinomycetes and its nature from Rothang Hill soil against viridans *Streptococcus* sp., *Res. J. Microbiol*. 2009; 1-6.
6. Schoenian, I., Spiteller, M, M., Manoj M, J. Chemical basis of the synergism and antagonism in microbial communities in the nests of leaf cutting ants. Proceedings of the National Academy of Sciences of the United States. *Int J Curr Microbiol App Sci*. 2014; 3: 801-32.
7. Selvameenal L., Radakrishnan M., Balagurunathan R. Antibiotic pigment from desert soil actinomycetes; biological activity, purification and chemical screening, *Indian J Pharma Sci*. 2009; 71: 499- 504.
8. Shimizu, M., Nakagawa, Y., Sato, Y., Furumai T., Igaroshi Y., Onaka H., Yoshida, R., and Kunoh, H., Studies on endophytic actinomycetes *Streptomyces* sp. isolated from Rododendron and its antifungal activity. *J Gen Plant Pathol* 2000; 66:360-66
9. Shirling, E.B. and Gottlieb, D., Methods for characterization of Streptomyces sp. Int. J. Syst. Bacteriol., 1966;16: 313-340.
10. Tinatin, D., and Nurzat, T., Biodiversity of Streptomyces of high-mountainous ecosystems of Kyrgystan and its biotechnological potential. *Antonie Leeuwenhoek* 2006;89:325-28
11. Thenmozhi M., Krishnan K. Anti-Aspergillus activity of Streptomyces sp. VITSTK7 isolated from Bay of Bengal coast of Puducherry, India. *J Nat Environ Sci*. 2011; 2(2): 1-8.
12. Vaijayanthi, G., Cholarajan, A. and Vijayakumar, R. Isolation, Characterization and Antibacterial Activity of Terrestrial Actinobacteria in the soils of Thanjavur District, Tamil Nadu, India. *Research J. Science and Tech.* 2012; 4(3): 132-139
13. Valan arasu N., Duraipandiyan M., Agastian M., Ignacimuthu S. Characterization and phylogenetic analysis of novel polyene type antimicrobial metabolite producing actinomycetes from marine sediments: Bay of Bengal India. *Mycol. Med*. 2008; 18:147- 153.
14. Vijayakumar, R., Muthukumar, C., Thajuddin, N., Panneerselvam, A and Saravanamuthu, R., Studies on the diversity of actinomycetes in the Palk Strait region of Bay of Bengal, India. Actinomycetologica, 2007; 21(2): 59-65: 0972 – 1487.

15. Vimal V., Rajan B.M., Kannabiran K. Antimicrobial activity of marine actinomycete, *Nocardiopsis* sp. VITSVK 5 (FJ973467). *Asian J Med Sci.* 2009; 1(2): 57-63.
16. Yang, L., Xie, J., Jiang D., Li, .G. and Lin, F., Antifungal substances produced by *Penicillium oxalicum* strain PY-2 potential antibiotics against plant pathogenic fungi. *World J Microbial Biotechnol* 2007; 24:909-15.
17. Yuan L., Zhang Y., Yu L., Sun C., Wei Y., Liu H., Li W., Zhang Y. Actinopolymorpha Cephalotaxi sp. nov., a novel actinomycete isolated from rhizosphere soil of the plant Cephalotaxus fortune. *Int J Syst Evol Microbiol.* 2010; 60: 51-54.