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# Isolation and screening of antimicrobial activity of some actinomycetes from agricultural soils of selected places from Warangal District, Telangana

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## ABSTRACT

One of the major problems in agriculture is the damage caused by phytopathogenic fungi. To minimize this damage and to control phytopathogenic fungi several conventional methods have been carried out by using synthetic fungicides but as their continuous application causes harm to human, animal, and environment, there is a need to search for an alternative to decrease the use of synthetic fungicides and to increase food production in agriculture. Using microorganisms as biocontrol agents is in long practice but in recent years actinomycetes have been widely used to control phytopathogenic fungi. However, actinomycetes have been isolated from different environments and their compounds and secondary metabolites have been studied extensively, in the present study we tried to isolate actinomycetes from unexplored sources and screened their antimicrobial activity in order to use them for the control of plant diseases.

**Keywords** phytopathogenic fungi, actinomycetes, antimicrobial activity, fungicides.

## INTRODUCTION

India with 195 mha under cultivation is a global agricultural powerhouse. It is the second largest producer of rice, wheat, cotton, sugarcane. 55.49% of Telangana population is dependent on agriculture & allied sectors for their livelihoods.

One of the major problems in agriculture is the damage caused by phytopathogenic fungi [1]. To minimize this damage and to control phytopathogenic fungi several conventional methods have been carried out by using synthetic fungicides; however, their continuous application causes resistance in microorganisms and causes harm to human, animal, and environment [2]. The search for an alternative to decrease the use of synthetic fungicides in agriculture and to increase food production efficiency is of global priority [3].

In recent years, the use of actinomycetes as a biocontrol agent on phytopathogenic fungi has been an alternative to the application of synthetic fungicides [4]. They have acquired prominence in recent years because of their antibiotic capacity. Actinomycetes are a group of microbes widely distributed across the world's natural ecosystems and are especially valuable for their organic cycling role [5,6]. Actinomycetes are gram-positive and slow-growing

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bacteria, distinguished by the development of aerial mycelium. 90% of actinomycetes genera have been isolated from soil, which are useful in different fields such as industrial, pharmaceutical and agriculture sectors [7].Actinomycetes, the heterogeneous group of microorganisms are a good source of unique bioactive metabolites, including antibiotics, enzymes, and plant growth factors [8, 9, 10].

To control phytopathogenic fungi actinomycetes exhibits one of the antagonistic mechanisms such as competence for space and nutrients [11], production of antibiotics [12], siderophores [13], lytic enzymes [14], volatile organic compounds (VOCs) [15], and host resistance induction [16]. Additionally, actinomycetes promote plant growth and development by fixing the atmospheric nitrogen or through the synthesis of phytohormones[17]. However, several studies have been done on actinomycetes; but very few studies have focused on actinomycetes isolation from different environments and their use as biocontrol agents in agriculture to control phytopathogenic fungi [18]. In the present study we isolated actinomycetes from various agricultural soils and screened for their antimicrobial activity.

## MATERIALS AND METHODS

### i. Soil Sample Collection

Hundred soil samples from different agricultural fields of Warangal district like rice, maize, cotton, ground nut etc., were processed during the research. The samples were named with location and field such as WglR, WglC, WglM and so on with increasing order of their altitude. Similarly, the isolates from each respective sample were labelled as WglR1, WglR2, and so on. The soil was collected within the depth of 10–12.5 cm. The samples were collected in sterile polythene bags, closely tightened, and were taken to the laboratory.

### ii. Isolation of Actinomycetes

One gram of soil sample was taken and serially diluted up to $10^{-6}$ using distilled water as a diluent. The mixture was shaken vigorously using a vortex; 0.1 ml of each dilution was placed on starch casein agar (composition: soluble starch: 10 g, $K_2HPO_4$: 2 g, $KNO_3$: 2 g, casein: 0.3 g, $MgSO_4.7H_2O$: 0.05 g, $CaCO_3$: 0.02 g, $FeSO_4.7H_2O$: 0.01 g, agar: 15 g, and filtered sea water: 1000 ml and pH: 7.0 ± 0.1), and the inoculum was spread properly using a sterile glass spreader. The inoculated plates were allowed to stand at room temperature for 5–10 minutes to allow the liquid to be absorbed and were incubated at 28°C for 7 days.

### iii. Morphological studies

The pure isolates of actinomycetes plates were prepared and after placing 3 to 4 sterile coverslips, the plates were incubated at 28 ± 2 °C for 4 to 7 days. The coverslips were removed at 3 days of interval and observed under the high power magnification. The arrangement of conidiospores on aerial and substrate mycelia was observed and compared with the Bergeys Manual of Determinative Bacteriology [19]. Cultural characteristics were studied by growing the organism on Luria Bertani Agar Media , Mannitol Malt yeast Extract ,Starch casein agar after 14 days of incubation at 28 °C.

### iv. Primary Screening:

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Primary screening of actinomycetes was performed on the Mueller–Hinton agar medium employing the perpendicular streak method. In the sterile agar medium, the pure isolate of actinomycetes was streaked along the diameter of the plate. The plate was incubated at 28°C for 7 days. Pure colony of test bacteria *Staphylococcus aureus* (ATCC 25923), *E. coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Klebsiella pneumoniae* (ATCC 700603) was transferred into fresh nutrient broth and incubated at 37°C for 24 hours until the visible turbidity. After adjusting the turbidity equal to that of 0.5 McFarland with the cell count of $1.5 \times 10^8$, the test organisms were streaked perpendicular to the isolate. The plates were further incubated at 37°C for 24 hours, and the antimicrobial activity was estimated [20].

**v. Secondary Screening:**

**Agar well diffusion method:**

Five wells of 6 mm diameter were made on the agar plate with the help of sterile cork borers. The test organism was swabbed on the agar surface and 100 $\mu$L of crude extract was poured in the wells. Ethyl acetate and antibiotic discs (oxacillin, nitrofurantoin, and ciprofloxacin) were used for negative and positive control, respectively. The plates were allowed to stand for few minutes and incubated at 37°C without inverting for 24 hours.

## RESULTS AND DISCUSSIONS

As shown in Table 1 a total of 15 isolates out of 100 soil samples were identified as actinomycetes species based on morphology and microscopic observation. All isolates were slow growing, aerobic, gram-positive nature with white to cream and greyish colour colonies.

**Table 1. Positive number of actinomycetes in collected soil samples**

| Location | No of samples | No of positive samples | |
|---|---|---|---|
| | | No | % |
| Wgl (Warangal) | 10 | 4 | 40 |
| Kk (Kadipikonda) | 20 | 3 | 15 |
| Ch (Chintagattu) | 5 | - | -- |
| Has (Hasanparthy) | 15 | 2 | 13.3 |
| Sp (Shambunipet) | 30 | 4 | 13.3 |
| Mn (Mamunoor) | 10 | 1 | 10 |
| Mk (Madikonda) | 4 | -- | -- |
| Bp (Bhattupalli) | 6 | 1 | 16.6 |
| Total | 100 | 15 | |

Among all those strains KkC 1, KkC 2, WglC 3, SpC 2, SpM 2 and MnC 1 showed the positive results for Primary screening.

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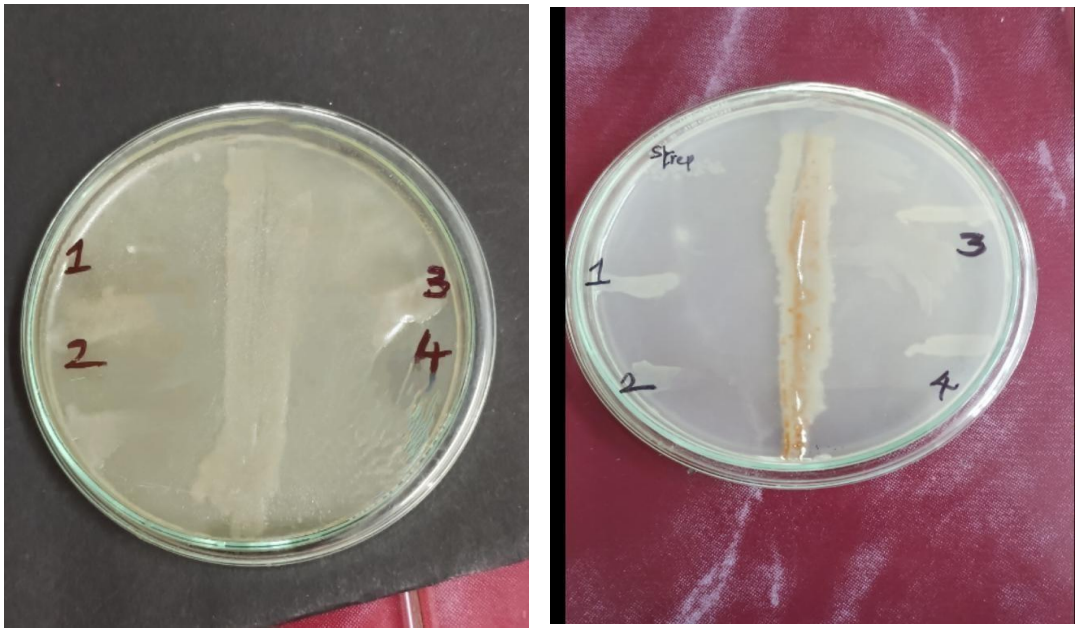


**Figure 1: Primary screening of an isolates KkC 2 and WglC 3**

Majority of the isolates exhibited antimicrobial activity against the tested organisms with varying zones of inhibition. The results are presented in Table 2. Among all the isolates KkC 2 and WglC 3 exhibited broad range of antimicrobial activity, whereas MnC 1 isolate showed high activity against gram negative organisms. As the two isolates KkC 2 and WglC 1 can be effectively used as bio control agents against pathogens. Hence, they were selected for further study.

**Table 2. Secondary screening of actinomycetes isolates**

| Isolate code | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | *B.subtilis* | *S.aureus* | *E.coli* | *K.pneumoniae* |
| WglR 1 | 5.0 ± 0.2 | 6.2 ± 0.2 | -- | 3.2 ± 0.2 |
| WglR 2 | -- | 2.5 ± 0.2 | 3.5 ± 0.2 | 2.9 ± 0.2 |
| WglC 1 | 9.5 ± 0.2 | 7.2 ± 0.2 | 4.5 ± 0.2 | 6.2 ± 0.2 |
| WglM 1 | 5.4 ± 0.2 | 6.4 ± 0.2 | 5.0 ± 0.2 | 3.9 ± 0.2 |
| KkC 1 | 7.0 ± 0.2 | 5.5 ± 0.2 | 3.0 ± 0.2 | 7.9 ± 0.2 |
| KkC 2 | 10.2 ± 0.2 | 8.0 ± 0.2 | 4.8 ± 0.2 | 4.0 ± 0.2 |
| KkM 1 | 6.5 ± 0.2 | 5.4 ± 0.2 | 4.6 ± 0.2 | -- |
| HasC 1 | -- | 2.0 ± 0.2 | 3.0 ± 0.2 | 2.5 ± 0.2 |
| HasC 2 | 2.5 ± 0.2 | 3.7 ± 0.2 | 4.8 ± 0.2 | 3.6 ± 0.2 |
| SpC 1 | 4.5 ± 0.2 | 5.2 ± 0.2 | 2.4 ± 0.2 | 2.0 ± 0.2 |
| SpC 2 | 5.3 ± 0.2 | 6.4 ± 0.2 | 3.7 ± 0.2 | 5.6 ± 0.2 |
| SpM 1 | -- | -- | 2.5 ± 0.2 | 3.2 ± 0.2 |
| SpM 2 | 6.9 ± 0.2 | 5.5 ± 0.2 | -- | 2.5 ± 0.2 |
| MnC 1 | 2.6 ± 0.2 | 5.9 ± 0.2 | 8.5 ± 0.2 | 9.2 ± 0.2 |
| BpM 1 | 4.5 ± 0.2 | 4.6 ± 0.2 | 4.4 ± 0.2 | 3.5 ± 0.2 |

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WglR-Wgl Rice field; WglC-Wgl Cotton field; WglM-Wgl Maize field; KkC-Kk Cotton field; KkM-Kk Maize field; HasC-Has Cotton field; SpC-Sp Cotton field; SpM-Sp Maize field

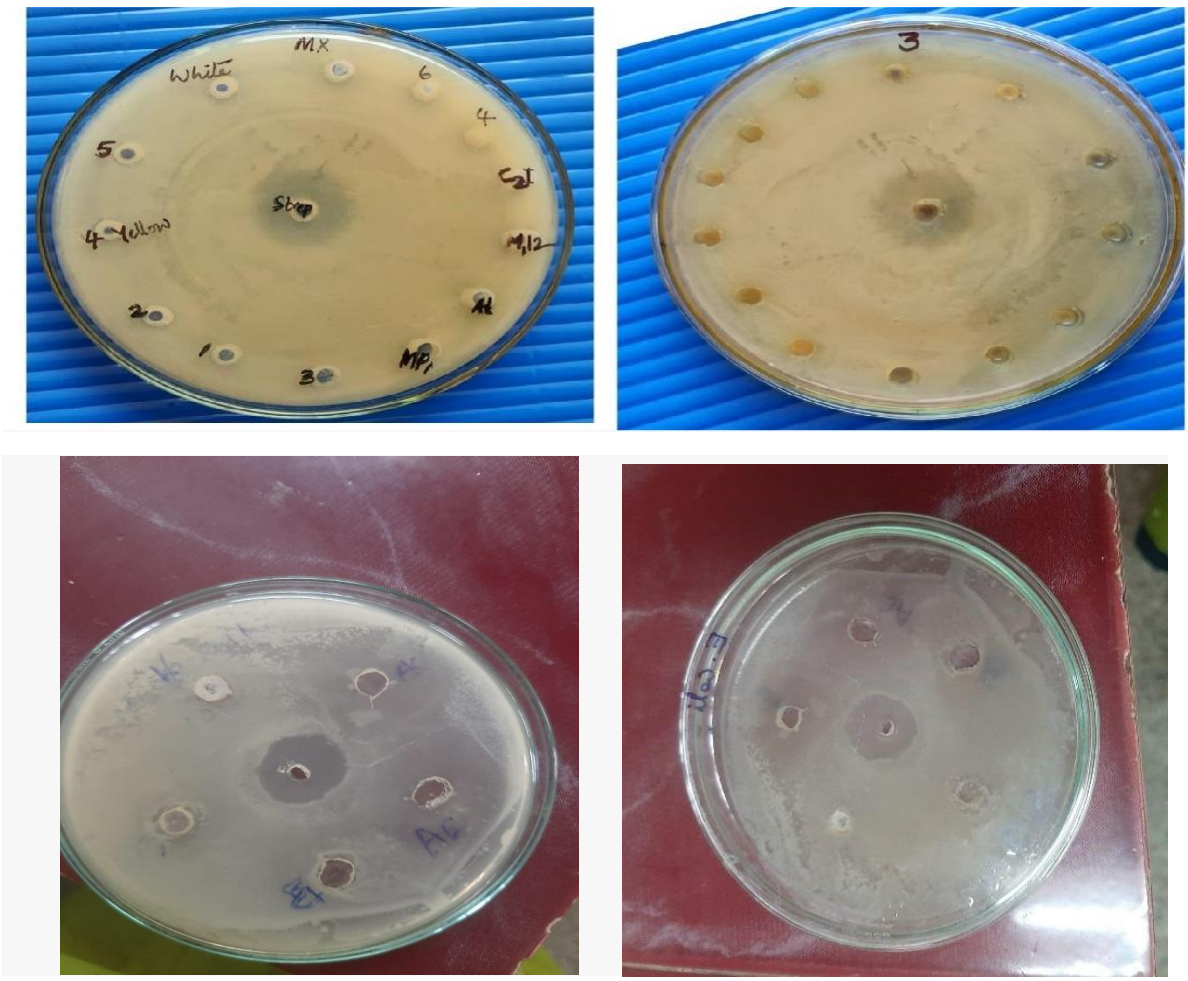

**Figure 2: Secondary screening of actinomycetes isolates**

## CONCLUSION

We can conclude that the soil samples are rich source of actinomycetes organisms which exhibit a wide spectrum antimicrobial activity and can be effectively used as biocontrol agents. Further investigations are needed to identify the strain at molecular level and to determine the active metabolites of these isolates.

## REFERENCES

1) Alblooshi, A.A.; Purayil, G.P.; Saeed, E.E.; Ramadan, G.A.; Tariq, S.; Altaee, A.S.; El-Tarabily, K.A.; AbuQamar, S.F. Biocontrol potential of endophytic actinobacteria against Fusarium solani, the causal agent of sudden decline syndrome on date palm in the UAE. J. Fungi 2022, 8, 8.
2) Torres-Rodriguez, J.A.; Reyes-Pérez, J.J.; Castellanos, T.; Angulo, C.; Quiñones-Aguilar, E.E.; Hernandez-Montiel, L.G. A biopoly- mer with antimicrobial properties

and plant resistance inducer against phytopathogens: Chitosan. Not. Bot. Horti Agrobot. Cluj-Napoca 2021, 49, 12231.

3) Xiao, L.; Niu, H.-J.; Qu, T.-L.; Zhang, X.-F.; Du, F.-Y. Streptomyces sp. FX13 inhibits fungicide-resistant Botrytis cinerea in vitro and in vivo by producing oligomycin A. Pestic. Biochem. Physiol. 2021, 175, 104834.
4) Boukaew, S.; Yossan, S.; Cheirsilp, B.; Prasertsan, P. Impact of environmental factors on Streptomyces spp. metabolites against Botrytis cinerea. J. Basic Microbiol. 2022, 62, 611–622.
5) Currie, C.R., Poulsen, M., Mendenhall, J., Boomsma, J.J., Billen, J., 2006. Coevolved crypts and exocrine glands support mutualistic bacteria in fungus-growing ants. Sci. 311, 81-83.
6) Javed Z., Tripathi G.D., Mishra M., Dashora K. Actinomycetes the microbial machinery for the organic-cycling, plant growth, and sustainable soil health. *Biocatal. Agric. Biotechnol.* 2020;101893.
7) Bawazir A. M. A., Shantaram M. Ecology and distribution of actinomycetes in nature—a review. *International Journal of Current Research.* 2018;**10**(7):71664–71668. doi: 10.24941/ijcr.31442.07.2018.
8) Nanjwade B., Chandrashekhara S., Goudanavar P., Shamarez A., Manvi F. Production of antibiotics from soil-isolated actinomycetes and evaluation of their antimicrobial activities. *Trop. J. Parm. Res.* 2010;9
9) Dimri A.G., Chauhan A., Aggarwal M.L. Antibiotic potential of actinomycetes from different environments against human pathogens and microorganisms of industrial importance: a review. *Sci Arc.* 2020; 1:32–48.
10) Gunasinghe Y.H.K.I.S., Edirisinghe E.A.A.D. Industrially important enzyme and plant growth promoter potential of soil Actinomycetes. *IJRASB.* 2020; 7:54–62.
11) Fadhilah Q.G., Santoso I., Maryanto A.E., Abdullah S., Yasman Y. Evaluation of the antifungal activity of marine actinomycetes isolates against the phytopathogenic fungi *Colletotrichum siamense* KA: A preliminary study for new antifungal compound discovery. *Pharmacia.* 2021; 68:837–843. doi: 10.3897/pharmacia.68.e72817.
12) Xia X., Liu J., Huang L., Zhang X., Deng Y., Li F., Liu Z., Huang R. Molecular details of actinomycin D-Treated MRSA revealed via high-dimensional data. *Mar. Drugs.* 2022; 20:114. doi: 10.3390/md20020114.
13) Meena L.I., Rajeswari E., Ahiladevi P., Kamalakannan A., Kalaiselvi T. Antifungal potential of *Streptomyces rameus* GgS 48 against mungbean root rot [*Rhizoctonia bataticola* (Taub.) Butler] *J. Biosci.* 2022; 47:10. doi: 10.1007/s12038-021-00244-5.
14) Gebily D.A.S., Ghanem G.A.M., Ragab M.M., Ali A.M., Soliman N.E.K., Abd El-Moity T.H. Characterization and potential antifungal activities of three *Streptomyces* spp. as biocontrol agents against *Sclerotinia sclerotiorum* (Lib.) de Bary infecting green bean. *Egypt. J. Biol. Pest Control.* 2021;31:33. doi: 10.1186/s41938-021-00373-x.
15) Gong Y., Liu J.-Q., Xu M.-J., Zhang C.-M., Gao J., Li C.-G., Xing K., Qin S. Antifungal volatile organic compounds from *Streptomyces setonii* WY228 control black spot disease of sweet potato. *Appl. Environ. Microbiol.* 2022; 88:e02317-21. doi: 10.1128/aem.02317-21.

16) Kaari M., Joseph J., Manikkam R., Sreenivasan A., Venugopal G., Alexander B., Krishnan S. Anti-Biofilm activity and biocontrol potential of streptomyces cultures against *Ralstonia solanacearum* on tomato plants. *Indian J. Microbiol.* 2022; 62:32–39. doi: 10.1007/s12088-021-00963-1.
17) Mitra D., Mondal R., Khoshru B., Senapati A., Radha T.K., Mahakur B., Uniyal N., Myo E.M., Boutaj H., Sierra B.E.G., et al. Actinobacteria-enhanced plant growth, nutrient acquisition, and crop protection: Advances in soil, plant, and microbial multifactorial interactions. *Pedosphere.* 2022; 32:149–170. doi: 10.1016/S1002-0160(21)60042-5.
18) Torres-Rodriguez JA, Reyes-Pérez JJ, Quiñones-Aguilar EE, Hernandez-Montiel LG. Actinomycete Potential as Biocontrol Agent of Phytopathogenic Fungi: Mechanisms, Source, and Applications. Plants (Basel). 2022 Nov 23;11(23):3201. doi: 10.3390/plants11233201. PMID: 36501241; PMCID: PMC9736024.
19) Arifuzzaman M., Khatun M.R., Rahman H. Isolation and screening of actinomycetes from Sundarbans soil for antibacterial activity. *Afri. J. Biotech.* 2010;9(29):4615–4619.
20) Shomura T., Omoto S.M., Ohba K., Ogino H. SF-1961, a new antibiotic related to bleomycin. *J. Antibiot.* 1980;33(11):1243–1248.