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ANTAGONISTIC ACTIVITY OF THE *STREPTOMYCES* SPECIES ISOLATED FROM RHIZOSPHERIC SOIL OF SELECTED MEDICINAL PLANTS

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Abstract: A total eleven *streptomyces* species were isolated from the plant rhizosphere soils. Extract were prepared by using Mineral medium and tested for their antibacterial activity against the Enterobacteriaceae members. Seven isolates (63%) of the *streptomyces* extracts were active against five pathogenic bacteria. 5 isolates (45%) of the *Streptomyces* extracts doesn't showed any activity against the 5 pathogenic organisms. 80% of the inhibition was showed by strain no 6 & 10 in the organisms *E. coli*, *K. pneumoniae* and *S. flexineri*. 70-75% of inhibition was showed by strain no 3, 4, 5 and 11 on the organisms *K. pneumoniae, S. typhi, E. coli* and *S. flexineri*.

Keywords: Medicinal Plants, Rhizospheric Soil, Actinomycetes, Antagonistic Activity, Enterobacteriaceae

INTRODUCTION

The region of contact between root and soil, where the soil is affected by roots was designated the rhizosphere. The rhizospheric microorganisms play important role. India has one of the richest plant medical cultures in the world. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers 'all' plants as potential sources of medicinal substances. The diversity and composition of bacterial taxa in the rhizosphere can be affected by several factors including plant species[16], soil type[7], soil management practices[17], microbial interaction[6] and other environmental variables. The composition of bacterial community in the rhizosphere is important for the performance of the plant, as bacterial species can have beneficial, neutral or harmful relationships with the roots[1,2,19].

Microorganisms have been intentionally introduced in to soil and rhizosphere environments in attempts to enhance certain agriculturally beneficial activities such as improvement of aggregate stability[12], suppression of plant pathogen[13] and promotion of plant growth[11]. Hence the study was planned to find out the rhizobacterial association of medicinal plants and the effect of rhizobacterial inoculum on antibacterial activity of the selected bacteria. Microorganisms have been shown to be attractive sources of natural compounds for the pharmaceutical and other industries actinomycetes are gram-positive bacteria. They are the most widely distributed group of microorganisms in nature. They are also well known as saprophytic soil inhabitants[20]. Most Actinomycetes in soil belong to the genus *Streptomyces*[3] and 75% of biologically active compounds are produced by this genus.

Actinomycetes occur in the plant rhizosphere soil and produce active compounds[18]. Attention has been paid to the possibility that Actinomycetes can protect roots by inhibiting the development of potential fungal pathogens by producing enzymes which degrade the fungal cell wall or producing antifungal compounds[4]. For example, *Streptomyces* spp. Strain 5406 has been used in China to protect cotton crops against soil-borne pathogens[21].

Actinomycetes can promote plant growth by producing promoters such as indole-3-acetic acid (IAA) to help growth of roots or produce siderophores to improve nutrient uptake[15]. However, the rate of discovery of new secondary metabolites has been decreasing, so the discovery of Actinomycetes in several sources increases the chance for the discovery of new secondary metabolites[5].

Active Actinomycetes may be found in medicinal plant root rhizosphere soils and may have the ability to produce new inhibitory compounds. The isolation of novel *Streptomyces* species is in great need as they are very potent producers of secondary metabolites. Many bacteria are intimately associated with plant roots. Rhizodeposition of various exudates provide an important substrate for the soil microbial community and there is a complex interplay between this community and the quantity and type of compounds released[9].

MATERIALS AND METHODS

Streptomyces spp. were isolated from rhizosphere soils of medicinal plants such as, *Azadiracta indica*, *Catharanthus roseus, Calotropis gigantia, Ocimum*

sanctum Linn collected in Gajuwaka, Visakhapatnam, Andhra Pradesh, India.`

Isolation of *Streptomyces* spp: Rhizosphere soil is collected from the rhizosphere zone of medicinal plants; soil is incubated at 60°C for 40min to remove the moisture content. 1gm of soil is suspended in 10ml of saline, mixed well and re-suspended in 40ml of saline, shaken for 30 min at 28°C[10]. The medium is supplemented with Nystatin (400mg/L) after sterilization to avoid fungal contamination. Starch casein agar plates were incubated for 14 days at30°c and the resulting colonies are sub cultured on Yeast extract- Malt extract agar medium.

Morphological and biochemical identification of isolated strains: A total of 11 strains were isolated and their morphological studies were studied by using light microscope and biochemical characters were studied by using standard biochemical methods for the identification and listed in Table no.1 & 2.

Table: 2 Biochemical characters:

Isolates	Indole	MR	VP	Citrate	catalase	oxidase	starch
Strain1	-	+	-	+	+	+	+
Strain2	-	+	-	+	+	+	+
Strain3	-	+	-	+	+	+	+
Strain4	-	+	-	+	+	+	+
Strain5	-	+	-	+	+	+	+
Strain6	-	+	-	+	+	+	+
Strain7	-	+	-	+	+	+	+
Strain8	-	+	-	+	+	+	+
Strain9	-	+	-	+	+	+	+
Strain10	-	+	-	+	+	+	+
Strain11	-	+	-	+	+	+	+

Table.1: Morphological characters:

S. no	Organism	Mycelium and nature of colony	Color of mycelium	Type of spore	Pigmentation	Gram stain
1	Strain 1	Septate, branched, Colored aerial mycelium	grey	Monosporophore	Yellow	+
2	Strain 2	Extensively branched, Aerial substrate Mycelium	Ash	Short chain of Spores	Brown	+
3	Strain 3	Smooth, granular Aerial &substrate Mycelium	White pink	Long chain spore	Wine red	+
4	Strain 4	Smooth, spirally twisted mycelium	White	Short chain spore	White	+
5	Strain 5	Smooth, hairy, raised wrinkled Aerial mycelium	White	Long chain spore	Yellow	+
6	Strain6	branched, septate Colored aerial mycelium	grey	Monosporophore	yellow	+
7	strain7	Smooth, granular Aerial &substrate Mycelium	Pink	Long chain spore	Wine red	+
8	Strain 8	Smooth, hairy, raised wrinkled Aerial mycelium	White	Long chain spore	yellow	+
9	Strain 9	Smooth, hairy, raised, wrinkled Aerial mycelium	White	Short chain spore	yellow	+
10	Strain 10	Smooth, granular Aerial &substrate Mycelium	Pink	Long chain spore	Wine Red	+
11	Strain 11	Smooth, hairy raised wrinkled Aerial mycelium	White	Short chain spore	yellow	+

Table.3: Antagonistic activity of *Streptomyces* spp:

S.no	Strain no.	Name of the organism				
		Zone of inhibition in percentage (%)				
		Klebsiella pneumoniae	*Salmonella typhii*	*Enterobacter aerogenes*	*Escherichia coli*	*Shigella flexineri*
1	Strain 1	65	60	65	60	60
2	Strain 2	-	-	-	-	-
3	Strain 3	-	75	-	70	-
4	Strain 4	**70**	65	65	60	60
5	Strain 5	**70**	60	60	65	**70**
6	Strain 6	**75**	**70**	-	**80**	-

7	Strain 7	-	-	-	-	-
8	Strain 8	-	-	-	-	-
9	Strain 9	-	-	-	-	-
10	Strain 10	**80**	65	65	**75**	**80**
11	Strain 11	-	70	-	-	-
12	Antibiotic (Ampicillin)	95	55	95	90	90

(-) indicates NILL (Zone of inhibition)

Preparation of crude extract: Crude extract of *Streptomyces* spp is prepared by inoculating in to 100 ml of mineral seed medium, composed of[8] starch 1% (w/v), glucose0.5% (w/v), yeast extract 0.2% (w/v), tryptone 0.5% (w/v), K_2HPO_4 1% (w/v), $MgSO_4.7H_2O$ 0.5% (w/v). The p^H was adjusted to 7.0 and incubated at 28°c for 3 days. The seed medium is transferred to 1000 ml of mineral medium and incubated for 72hrs at 30°c with constant shaking.

Crude product recovery: The fermented broth was centrifuged at 8000 rpm for 10 min. The filtrate was collected and used to test its antibacterial activity against selected Enterobacteriaceae members *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Salmonella typhii* ATCC 6539, *Shigella flexineri* ATCC 29508, *Enterobacter aerogens* ATCC 13048.

Assay for antibacterial activity: The standard agar diffusion method was followed for the assay of antibacterial activity of the crude extract. Muller-Hinton agar plates were prepared along with the test organism by pour plate method. After solidification wells are prepared by gel borer of 6mm size. Cups are filled with 50µl of the crude extract each. A broad spectrum antibiotic of Ampicillin was used as reference standard (100units/100ml).

The plates were incubated at 28°c ±2 for 24 hrs without inverting the plates. The zones were measured by standard Hi Antibiticaz Zone Scale-^{TM}CPW 297 (Supplied by HIMEDIA). The zone of inhibition was expressed in percentage (%) in table.3.

RESULTS AND DISCUSSION

Seven isolates (63%) of the *streptomyces* extracts were active against the five pathogenic bacteria. Five isolates (45%) of the *streptomyces* extracts doesn't showed any activity against the five pathogenic organisms. From the total of 7 active *streptomyces* 5 extracts showed their activity on all the selected Enterobacteriaceae members.80% of the inhibition was showed by strain no 6 & 10 on the organisms *E. coli, K. pneumoniae* and *S. flexineri*. 70 to 75% of the inhibition was showed by strain no 3, 11, 5, 4 on the organisms *K. pneumoniae, S. typhi, E. coli* and *S. flexineri*.60 to 65% of the inhibition was showed by all the active strain extracts on all the pathogenic bacteria.

Fig.1: Showing antibacterial activity of *Streptomyces* isolates:

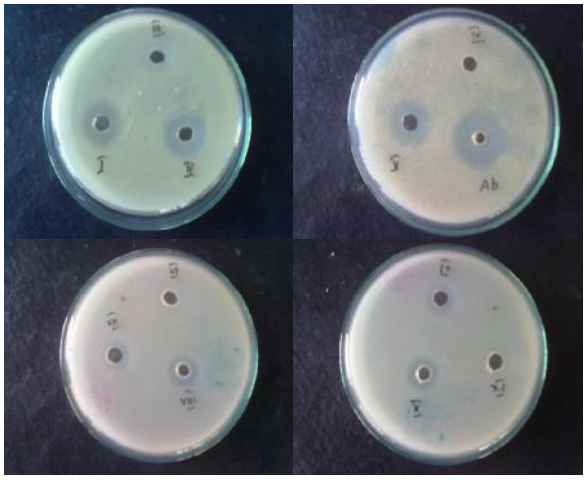


Fig 2: Showing % Of Inhibition of *Streptomyces* spp On *Shigella flexineri*

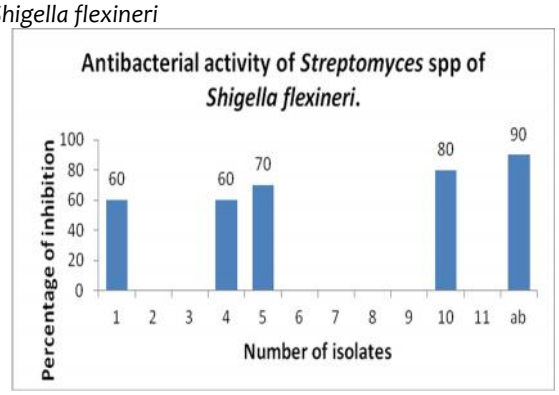


Fig 3: Showing % Of Inhibition of *Streptomyces* spp On *Klebsiella pneumoniae*

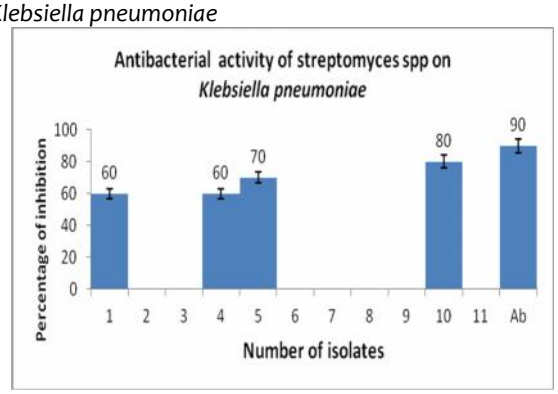


Fig 4: Showing % Of Inhibition of *Streptomyces* spp On *Salmonella typhii*

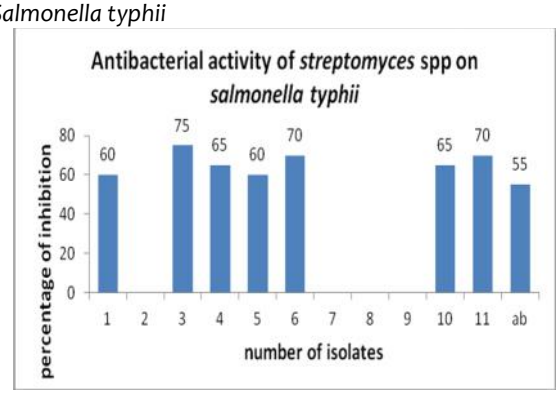


Fig 5: Showing % Of Inhibition of *Streptomyces* spp On *Enterobacter aerogens*

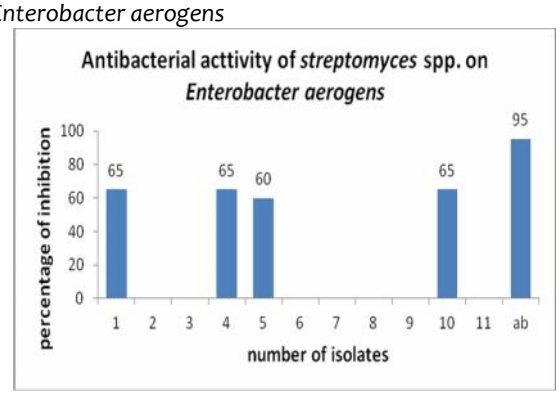
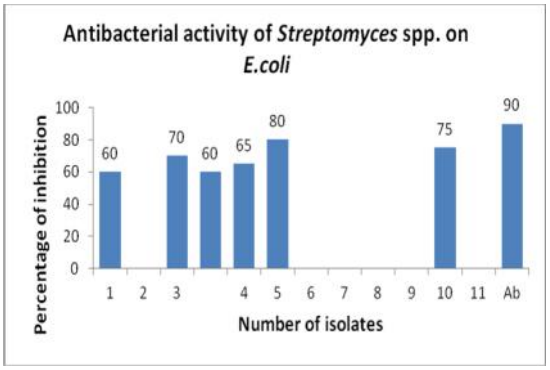


Fig 6: Showing % of inhibition Of *Streptomyces* spp On *Escherichia coli*



CONCLUSION

Among the 11 extracts, 4 extracts were shows their maximum activity on all the selected members. 3 extracts shows their activity only on *Salmonella typhii, Escherichia coli* and *Klebsiella pneumoniae*.5 extracts doesn't show any activity on all the selected Enterobacteriaceae members. Most of the Actinomycetes utilize root exudates for growth and synthesis of antimicrobial substances. Active Actinomycetes found in medicinal plant root rhizosphere soils and have the ability to produce new inhibitory compounds. The discoveries of Actinomycetes in several sources increase the chance for the discovery of new secondary metabolites.

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REFERENCES

1. Atkinson D and CA Watson, The beneficial rhizosphere, a dynamic entity. Appl. Soil Ecol., 15, 99-104 (2000).
2. Buchenauer H, Biological control of soil borne diseases by rhizobacteria, J. Plant Dis. Protect., 105, 329-348 (1998).
3. Goodfellow M, Simpson KE, Ecology of *Streptomycetes*. Front Appl Microbiol, 1987, 2:97–125.
4. Goodfellow M, Williams ST, Ecology of actinomycetes. Annu Rev Microbiol, 1983, 37:189–216.
5. Hayakawa M, Yoshida Y, Iimura Y, Selection of bioactive soil actinomycetes belonging to the Streptomyces violaceusniger phenotypic cluster. J Gen Appl Microbiol, 2004, 96:973–981.
6. Hedges RW and E Messens, Genetic aspects of rhizosphere interactions. In: The rhizosphere (Ed.: J.M. Lynch). Wiley, New York. 1990, 129-176.
7. Hoitink HAJ and MJ Boehm, Biocontrol within the context of soil microbial communities: A substrate dependent phenomenon, 1999.
8. Jayapradha Ramakrishnan, Murugesh Shunmuga sundaram, Mahesh Narayanan, Streptomyces sp. SCBT isolated from rhizospheric soil of medicinal plants is antagonistic to pathogenic bacteria: Iranian Journal of Biotechnology, 2009, vol. 7, No. 2.
9. Kandeler E, Marschner P, Tscherko D, Gahoonia TS, Nielsen NE, Microbial community composition and functional diversity in the rhizosphere of maize. Plant and Soil. 2002, 238: 301-312.
10. Kuster E, Willaims S, Selection of media for isolation of *Streptomycetes*. *Nature,* 1964, 202: 988-929.
11. Lambert B and H Joos, Fundamental aspects of rhizobacterial plant growth promotion research. TIBTECH, 1989, 7, 215-219.

12. Lynch JM, Promotion and inhibition of soil aggregate stability by microorganisms. J. Gen. Microbiol., 1981, 126, 371-375.

13. Maplestone PA and R Campbell, Colonization of roots of wheat seedlings by Bacilli proposed as biocontrol against take all. Soil Biol. Biochem, 1989, 21, 543-550.

14. Mellouli L, Ameur-Mehdi RB, Sioud S, Salem M, Bejar S, Isolation, purification and partial characterization of antibacterial activities produced by a newly isolated *Streptomyces* sp. US24 strain. *Res* Microbiol. 2003, 154: 345-352.

15. Merck R, Dijkra A, Hartog AD, Veen Jav, Production of root-derived material and associated microbial growth in soil at different nutrient levels. Biol Fertil Soils, 1987, 5:126–132.

16. Miller HJ, G Henken and JA VanVeen, Variation and composition of bacterial populations in the rhizosphere of maize, wheat and grass cultivars. Can. J. Microbiol., 1989, 35, 656-660.

17. Rovira AD, LF Elliot and RJ Cook, The impact of cropping systems on rhizosphere organisms affecting plant health. In: The rhizosphere (Ed.: J. M. Lynch). Wiley, New York. 1990, pp. 389-436.

18. Suzuki S, Yamamoto K, Okuda T, Nishio M, Nakanishi N, Komatsubara S, Selective isolation and distribution of Actinomadura rugatobispora strains in soil. Actinomycetology, 2000, 14:27–33.

19. Sylvia DM and DO Chellemi, Interactions among root-inhabiting fungi and their implications for biological control of root pathogens. Adv. Agron., 2001, 73, 1-33.

20. Takisawa M, Colwell RR, Hill RT, Isolation and diversity of actinomycetes in the Chesapeake Bay. Appl Environ Microbiol, 1993, 59:997–1002.

21. Valois D, Fayad K, Barasubiye T, Garon T, Dery C, Brzezinski R, Beaulieu C, Glucanolytic actinomycetes antagonistic to Phytophthora fragariae var. rubi, the causal agent of raspberry root rot. Appl Environ Microbiol, 1996, 62:1630–1635.

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