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\(^6\), soil type\(^7\), soil management practices\(^7\), microbial interaction\(^8\) 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\(^13\), suppression of plant pathogen\(^13\) and promotion of plant growth\(^17\). 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\(^25\). 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\(^16\). 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\(^31\).

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\(^23\). 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

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

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MATERIALS AND METHODS

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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. The medium is supplemented with Nystatin (400mg/L) after sterilization to avoid fungal contamination. Starch casein agar plates were incubated for 14 days at 30°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>
<thead>
<tr>
<th>S. no</th>
<th>Organism</th>
<th>Mycelium and nature of colony</th>
<th>Color of mycelium</th>
<th>Type of spore</th>
<th>Pigmentation</th>
<th>Gram stain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Strain 1</td>
<td>Septate, branched, Colored aerial mycelium</td>
<td>grey</td>
<td>Monosporophore</td>
<td>Yellow</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Strain 2</td>
<td>Extensively branched, Aerial substrate Mycelium</td>
<td>Ash</td>
<td>Short chain of Spores</td>
<td>Brown</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Strain 3</td>
<td>Smooth, granular Aerial &amp;substrate Mycelium</td>
<td>White pink</td>
<td>Long chain spore</td>
<td>Wine red</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Strain 4</td>
<td>Smooth, spirally twisted mycelium</td>
<td>White</td>
<td>Short chain spore</td>
<td>White</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Strain 5</td>
<td>Smooth, hairy, raised wrinkled Aerial mycelium</td>
<td>White</td>
<td>Long chain spore</td>
<td>Yellow</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Strain6</td>
<td>branched, septate Colored aerial mycelium</td>
<td>grey</td>
<td>Monosporophore</td>
<td>yellow</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Strain7</td>
<td>Smooth, granular Aerial &amp;substrate Mycelium</td>
<td>Pink</td>
<td>Long chain spore</td>
<td>Wine red</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Strain 8</td>
<td>Smooth, hairy, raised wrinkled Aerial mycelium</td>
<td>White</td>
<td>Long chain spore</td>
<td>yellow</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Strain 9</td>
<td>Smooth, hairy, raised, wrinkled Aerial mycelium</td>
<td>White</td>
<td>Short chain spore</td>
<td>yellow</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Strain 10</td>
<td>Smooth, granular Aerial &amp;substrate Mycelium</td>
<td>Pink</td>
<td>Long chain spore</td>
<td>Wine Red</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>Strain 11</td>
<td>Smooth, hairy raised wrinkled Aerial mycelium</td>
<td>White</td>
<td>Short chain spore</td>
<td>yellow</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 3: Antagonistic activity of Streptomyces spp:

<table>
<thead>
<tr>
<th>S.no</th>
<th>Strain no.</th>
<th>Name of the organism</th>
<th>K. pneumoniae</th>
<th>S. typhimurium</th>
<th>E. aerogenes</th>
<th>E. coli</th>
<th>S. flexineri</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Strain 1</td>
<td>65</td>
<td>60</td>
<td>65</td>
<td>60</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Strain 2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Strain 3</td>
<td>-</td>
<td>75</td>
<td>-</td>
<td>70</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Strain 4</td>
<td>70</td>
<td>65</td>
<td>65</td>
<td>60</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Strain 5</td>
<td>70</td>
<td>60</td>
<td>60</td>
<td>65</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Strain 6</td>
<td>75</td>
<td>70</td>
<td>-</td>
<td>80</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

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Preparation of crude extract: Crude extract of Streptomyces spp is prepared by inoculating in to 100 ml of mineral seed medium, composed of 1%) starch 1% (w/v), glucose 0.5% (w/v), yeast extract 0.2% (w/v), tryptone 0.5% (w/v), K,HPO₄ 1% (w/v), MgSO₄·7H₂O 0.5% (w/v). The pH 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®CPW 297 (Supplied by HIMEDIA). The zone of inhibition was expressed in percentage (%) in table 3.

RESULTS AND DISCUSSION
Seven isolates (63%) of the streptomycys extracts were active against the five pathogenic bacteria. Five isolates (45%) of the streptomycys extracts doesn't showed any activity against the five pathogenic organisms. From the total of 7 active streptomycys 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.
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 typhi, 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


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