Preliminary screening of rhizospheric soil samples for the isolation of actinomycetes and determination of its antimicrobial activity

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Abstract:

In the current scenario mostly the researchers are interested in combating the resistant pathogens by trying to discover most effective secondary metabolites produced by the group of actinomycetes and other microorganisms. Present study aims to carry out preliminary investigation by screening the rhizospheric soil samples by crowded plate technique to isolate the microorganisms showing antagonistic activity. Out of the 10 selected cultures A1, A3, A6, A7, A8 and A9 showed antimicrobial activity against the standard test cultures. Spectral studies of the crude starch casein medium showed peaks in the range of 200-280nm for culture A6 and mostly at 245nm for other cultures. Except A1 others were found to be producing yellow pigment with the exception of A6 which produced brown pigment. Hence, it is concluded that cultures have the potential to produce antimicrobial compounds and have a vast scope for further studies.

Key words: actinomycetes, secondary metabolite, starch casein medium, crowded-plate technique, antimicrobial activity

Introduction:

Microbial pathogens are having peculiar mechanisms of developing resistance against existing antibiotics, thus stressing the need for the discovery of new therapeutic compounds. Mostly this potential has been observed in actinomycetes. They are Gram positive bacteria.
having high G+C (>55%) content in their DNA. Actinomycetes are the prokaryotes which tend to show hyphal morphology. The majority of actinomycetes are free living, saprophytic bacteria found widely distributed in soil, water and colonizing plants. Actinomycetes population has been identified as one of the major group of soil population which may vary with the soil type [1]. Most of the actinomyces are the incomparable sources of bio-active metabolites including antibiotics, plant growth factors and other substances[2]. They are mostly soil microorganisms but can be found in diverse habitats and are active in the decomposition of plant tissues and thereby in the recycling of carbon and nitrogen and are known to produce approximately 70-80% of the available antibiotics [3]. In the past several decades a large number of actinomycetes have been isolated and identified to produce secondary metabolites commercially available [4]. Isolation of rare actinomycetes using conventional methods is still a difficult task. Novel genera can be isolated by considering several factors which includes selection of ecological habitats for sample collection, chemical and physical pretreatment of the samples, use of specific selective media, fine-tuning of culture conditions and genus-specific methodologies [5,6]. Actinomycetes have gained great importance in the pharmaceutical industry for their unlimited capacity to produce secondary metabolites including antibiotics with diverse chemical structure and biological activities. Actinomycetes are used in the production of a diverse array of antibiotics including β-lactams, aminoglycosides, peptides, polyenes, polyether, tetracyclines, macrolides, etc. In searching for new antibiotics, over 1,000 different bacteria, actinomycetes, streptomyces, fungi and algae have been investigated. To prevent the emergence of multiple drug resistant microorganisms to the clinically available antibiotics already marketed, a periodic replace of the existing antibiotics is necessary[7]. Immense knowledge in the area of pathogen’s drug resistance has evoked the discovery of new antibiotics by the screening of microbes. At present, aerobic actinomycetes have attracted considerable attention of bacteriologist, geneticist and ecologist because of the production of novel antibiotics[8,9].
Materials and methods:

1. Collection of sample: Rhizospheric soil samples were collected from various locations of Aurangabad and air dried.

2. Screening and isolation of strains showing antagonistic activity: 10 grams of soil sample was suspended in 100 ml of sterile distilled water and kept on rotary shaker at 50 rpm in 100 ml of sterile distilled water and kept on orbital rotary shaker at 50 rpm for 15 min at room temperature. Serial dilutions were prepared up to $10^{-4}$, 0.1 ml of each dilution was spread on starch casein agar medium and kept for incubation at 30°C for 5 days. Colonies showing inhibition around it were selected and transferred on the slants of the same medium and refrigerated for further use.

3. Preliminary identification by cover slip technique: The sterile starch casein agar plates were inserted with sterile cover slips at an angle of 45° and the culture was streaked at the edge on the medium and incubated at 30°C for 7 days till the culture spreads on the cover slip. Cover slip was later on placed on the slide and observed under high power objective. Morphological characteristics were noted for the preliminary identification of the cultures.

4. Determination of antagonistic activity of the selected actinomycete cultures: Crude cultured broth was drawn out for each after specific time intervals, centrifuged at 10,000 rpm for 15 min and 50 µl of clear supernatant was poured in the 6mm well of antibiotic assay agar and checked for the antagonistic activity against the selected microorganisms ($Bacillus subtilis$ NCIM 2920, $Pseudomonas aeruginosa$ NCIM 2200, $Staphylococcus aureus$ NCIM 2079, $Escherichia coli$ NCIM 2065, $Aspergillus niger$ NCIM 596) by well diffusion method.

5. Spectral studies of the crude culture supernatant: The crude culture supernatant was used for spectral studies and identification of the nature of the antagonistic compound was done by considering the peaks at various wavelengths.
Results and discussion:

1. Screening for antagonistic activity by crowded plate technique.

Soil dilution was used to isolate actinomycetes by crowded plate technique. 10 cultures showing colony characteristics like actinomycetes and showing good zone of inhibition among several others were isolated and selected for detecting the antimicrobial activity against the standard test cultures by streak plate method. All the cultures showed antimicrobial activity towards few standard cultures.

2. Testing for the antagonistic activity against test cultures:

<table>
<thead>
<tr>
<th>Microbial test cultures</th>
<th>Bacillus subtilis 2920</th>
<th>Pseudomonas aeruginosa 2200</th>
<th>Staphylococcus aureus 2079</th>
<th>Escherichia coli 2065</th>
<th>Aspergillus niger 596</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture isolates ↓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>A2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>A3</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<td>A4</td>
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<td>A5</td>
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<tr>
<td>A6</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>A7</td>
<td>+</td>
<td>+</td>
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<td>A8</td>
<td>+</td>
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<td>A9</td>
<td>+</td>
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<tr>
<td>A10</td>
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</tr>
</tbody>
</table>

+ Inhibition in the range of 8mm-11mm, - No inhibition.

Cultures A1 and A6 showed antibacterial as well as antifungal activity whereas A3, A7, A8 and A9 showed only antibacterial activity. Preliminary investigation indicates that the culture has the potential to inhibit Gram-positive and Gram-negative bacteria. This indicates that the
antimicrobial activity of potential strain is due to the production of extracellular bioactive
compounds [10].

3. Determination of peaks by spectral studies by UV-Vis spectrophotometer:

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Cultures</th>
<th>Peaks (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>A1</td>
<td>245</td>
</tr>
<tr>
<td>2.</td>
<td>A3</td>
<td>245</td>
</tr>
<tr>
<td>3.</td>
<td>A6</td>
<td>222, 245*</td>
</tr>
<tr>
<td>4.</td>
<td>A7</td>
<td>244</td>
</tr>
<tr>
<td>5.</td>
<td>A8</td>
<td>247</td>
</tr>
<tr>
<td>6.</td>
<td>A9</td>
<td>245</td>
</tr>
</tbody>
</table>

*Various peaks were observed in the range of 220-250 nm, maximum absorption was observed at 222 and 245nm.

Peaks in the range of 220-250nm indicates that compound showing antimicrobial activity is
most probably a type of peptide antibiotic. S carneum have been reported to produce a
lipglycopeptide antibiotic complex with an activity against Gram-positive bacteria, a maximum
UV absorption at 282 nm [11]. S parvulus RSPSN2 was reported to show maximum absorption
at 242 nm which is very near to 245nm as has been observed [12,13]. Maximum absorbance
peaks ranging in between 200-295 nm indicates highly polyene nature which was observed with
culture A3[14].

4. Pigment production by isolates.

Pigment production was shown by all isolates except A1. Isolate A6 showed brown
pigmentation whereas others showed yellow pigments. Such pigment production has
been reported by other researchers.

Conclusion:

Amongst all the isolates six cultures were found to be showing antibacterial activity and two of
them have fungal activity also. Spectral studies of crude broth samples indicates that mostly the
bioactive compound is a peptide and one of them may have polyene nature. Preliminary studies indicates the potential of the microorganisms to produce antimicrobial compounds and hence can be used further for production, extraction and purification of the compounds and determination of its potency.

References:


