STUDIES ON ANTAGONISTIC ACTINOMYCETES FROM CASUARINA EQUISELIFOLIA L.

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Abstract:

The Rhizosphere soils are rich in microflora. A number of Actinomycetes were found associated with rhizosphere soils of *Casuarina* plants. A total of 20 different actinomycetes were isolated from the rhizosphere soil of *Casuarina equisetifolia* L. from different localities of Maharashtra. These included 14 species of *Streptomyces*, four species of *Streptoverticillium* and two unidentified Actinomycetes species. The Actinomycetes are rich source of antibiotics. The isolates were tested for their antagonistic properties against phyto-pathogenic and human pathogenic bacteria and fungi. *Streptomyces chattanoogensis* & *Streptomyces violaceochromogenes* inhibited the growth of *Bacillus subtilis* while *Streptomyces violaceochromogenes*, *Streptoverticillium fervens* and Actinomycete (1) were antagonistic to all phyto-pathogenic fungi. These experiments clearly indicated that actinomycetes isolated from rhizosphere of *Casuarina equisetifolia* L. show antagonism against the soil pathogens

Keywords: Actinomycetes, *Streptomyces*, Antagonism.

Introduction

The Rhizosphere soils are near to the plant roots and is rich in microflora. The Actinomycetes are one of group of microorganisms that are beneficial in nature and source of many antibiotics which are active against pathogenic organisms (Abhijit kulkarni, 2010). The antibiotic compounds are useful as human & veterinary medicine, in agriculture & biochemistry. It is a transitional group between simple bacteria & Fungi. They are gram positive & are classified under the order Actinomycetales (Bergey's Manual, 1989). Most of Microbial equilibrium is always maintained by the activity of these organisms.

The main economical role of Actinomycetes is as a decomposer of organic matter, help in carrying out many biochemical reactions in soil & increase the soil fertility. Numerically though they are less dominant than fungi but are potentially more active. They provide a granular & viable structure to soil for crop production. The various antibiotics produced by Actinomycetes selectively antagonise other soil microorganisms and help in maintaining microbial equilibrium (Williams, 1978). Genus *Frankia* grow in nodules of non leguminous trees and fix atmospheric nitrogen (Halbe & Nair, 2002).

Vegetative hyphae produce an extensively branched mycelium that rarely fragments. The aerial mycelium at maturity forms 3 to many spores. Initially colonies are relatively smooth surfaced but later they develop a web of aerial mycelium that may appear granular, powdery or velvety. They produce wide

range of pigments responsible for colour of vegetative & aerial mycelium. They may form colour diffusible pigments in the medium also (Kutzner 1981). Many actinomycetes use wide range of organic compound as a sole source of carbon for energy and growth at optimum temp 25-35°C, optimum pH range for growth at 6.5 – 8.0 (Pridham, 1974). The cell wall of Actinomycetes contain LL-diaminopimelic acid and DL-DAP (Lechevalier & Lechevalier, 1967).

Materials and Methods

For selective isolation of Actinomycetes, treatment of soil was essential. Therefore, 1 gm of soil was thoroughly dried at room temperature and mixed with 0.1 gm of calcium carbonate. It was incubated for 7 days at 26°C in water saturated environment. This treated soil was used for dilutions in phenol water solution (phenol: water 1:40). 0.1 ml of each dilution was used for each plate. The plates were incubated for 4-5 days.

Different media like starch-casein Agar, Inorganic salt starch Agar, MGA – SE Agar medium were used for isolation of actinomycetes and the Starch – Casein Agar and MGA-SE agar gave good results. After three days of incubation, actinomycete colonies started appearing on the medium. They were counted from the 5th day till 10 days. The colour and number of colonies were recorded.

Actinomycetes were identified upto the generic level with the help of slide & coverslip cultures (Walksman, 1961 ; Buchnar & Gibson, 1975). For identification of Actinomycetes upto

species level physiological & biochemical tests were carried out. They included growth on Yeast-Extract Malt Extract Agar, Glycerol asparagine agar etc. Utilization of carbon source was done using basal medium of Pridham (1974a) with different carbon sources. Colour determination was done by observation of mass colour of mature sporulating aerial surface growth, the colour of substrate mycelium as viewed from the reverse side of the plate and diffusible soluble pigments other than melanin. The substrate mycelium colour was identified by using Special guide on actinomycetes.

The strains of pathogenic bacteria and fungi were obtained from NCL, Pune and Department of Botany, BAMU, Aurangabad. Antagonistic tests of actinomycetes against bacteria and fungi were done using cross streak plate method of Huber & Watson (1966).

Results

Actinomycetes were isolated from Rhizosphere soils of *Casuarina* collected from different localities of Maharashtra. A total of 20 different actinomycetes were isolated from the rhizosphere soil of *Casuarina equisetifolia* L. from different localities like Pune, Ahmednagar, Kolhapur, Ratnagiri, Dapoli. These included 14 species of *Streptomyces*, four species of *Streptoverticillium* and two unidentified Actinomycetes species. They are broadly included six different groups based on colours. i.e. grey reverse colourless, grey reverse dark,

white reverse colourless, white reverse dark, pink and brown and further differentiated based on sugar utilization, carbon source and spore chain morphology (SEM).

Seven isolates were tested for antagonism against phyto-pathogenic and human pathogenic bacteria. The pathogens used were *Bacillus subtilis*, *Escherchia coli*, *Staphylococcus aureus*, *Salmonella paratyphi* and *Xanthomonas citri* and Nine isolates were tested for antagonism against phyto-pathogenic fungi *Alternaria alternata*, *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium sclerotiorum*.

All the isolates tested inhibited growth of *B. subtilis*. The actinomycetes *Streptomyces fervens*, *Streptomyces chattanoogensis* and *Streptomyces violaceochromogenes* inhibited the growth of *Bacillus subtilis* and *Escherchia coli* (Table I). Similarly *Streptomyces violaceochromogenes*, *Streptoverticillium fervens*, Actinomycete (1) were found to be antagonistic to all studied phyto-pathogenic fungi. Phytopathogenic fungi like *Alternaria alternata*, *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium sclerotiorum* were sensitive to the isolates tested like *Streptomyces violaceochromogenes*, *Streptoverticillium fervens*. (Table II). These experiments clearly indicated that all actinomycetes isolated from rhizosphere of *Casuarina equisetifolia* L. produced antibiotics which are mostly antagonistic to phyto-pathogenic fungi as well as pathogenic bacteria.

Table I - ANTAGONISM OF ACTINOMYCETES AGAINST PATHOGENIC BACTERIA

Sr. No	Name of the Actinomycete	<i>Bacillus subtilis</i>	<i>Escherchia coli</i>	<i>Xanthomonas citri</i>	<i>Staphylococcus aureus</i>	<i>Salmonella paratyphi</i>
1	<i>Streptomyces chattanoogensis</i>	++	++	--	--	--
2	<i>Streptomyces olivaciscieroticum</i>	++	--	++	--	--
3	<i>Streptomyces violaceochromogenes</i>	++	++	--	--	--
4	Actinomycete (1)	++	++	--	++	--
5	<i>Streptoverticillium heroshemense</i>	++	++	--	++	--
6	<i>Streptomyces fervens</i>	++	++	--	--	--
7	<i>Streptoverticillium lavenduligriseum</i>	++	++	--	--	--

Table II- ANTAGONISM OF ACTINOMYCETES AGAINST PATHOGENIC FUNGI

Sr. No	Name of the Actinomycete	<i>Alternaria alternata</i>	<i>Fusarium oxysporum</i>	<i>Rhizoctonia solani</i>	<i>Sclerotium sclerotiorum</i>
1	<i>Streptomyces chattanoogensis</i>	++	++	--	--
2	<i>Streptomyces olivaciscieroticum</i>	++	++	--	++
3	<i>Streptomyces violaceochromogenes</i>	++	++	++	++
4	Actinomycete (1)	++	++	++	++
5	<i>Streptoverticillium heroshemense</i>	--	++	--	--
6	<i>Streptomyces fervens</i>	++	++	++	++
7	<i>Streptoverticillium lavenduligriseum</i>	++	--	--	--
8	<i>Streptomyces finlayi</i>	--	++	++	++
9	<i>Streptomyces griseoruber</i>	--	++	++	--

Conclusion

These experiments clearly indicated that the Rhizosphere soils of *Casuarina equisetifolia* are rich in Actinomycetes. These isolates produced antibiotics that are active against phyto-pathogenic fungi and bacteria. This ability of the Rhizosphere make the plant roots sturdy and allow them spread and grow. Thus, the actinomycetes are potential organisms for production of antibiotics.

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