Original Research Article

IDENTIFICATION OF A NOVEL *STREPTOMYCES* SPECIES FOR MANAGEMENT OF PLANT DISEASE

ABSTRACT

Groundnut is a major oilseed crop around the world. It is also known as the 'King' of oilseeds. Though the groundnut is attacked by number of diseases, the soil borne fungal disease, stem rot caused by *Sclerotium rolfsii* is a potential threat to groundnut production and it causes yield losses over 25 % [1] Present study was planned to formulate a suitable groundnut stem rot management technology through isolation of location specific biocontrol agents and to investigate unexplored microorganisms for stem rot management as an alternate for existing biocontrol agents. In order to study the morphological characters of different isolates, the eight isolates were inoculated in the PDA medium and the characters were studied. The results indicated that the pathogen showed variations in their virulence as well as morphological characters. Among the hundred isolates tested GNRAJK1 and GNRAVR14 were found to be highly effective in inhibiting the mycelial growth of pathogen. The present study identified two unknown promising antagonists and they were found to be effective against *Sclerotium rolfsii*. Later the unknown antagonists were found to belong to genus *Streptomyces* based on the biochemical studies, gram staining, medium specific culturing etc. The other method like Thin Layer Chromatography was carried out to observe the composition of the cell wall aminoacid present in the antagonists and found that it contained LL-DAP (Diaminophimelic acid) along with glycine indicating the cell wall type -1which are prevalent in the group of *Streptomyces*. Further the isolate was investigated through Biolog analysis to reconfirm the promising stem rot antagonist and found that based on the colony character, growth pattern, medium specific culturing and spore forming character it was reconfirmed that the group is *Streptomyces*. Further, fatty acids methyl ester studies were conducted to identify the species by identifying 9 and 20 length carbon fatty acids and related compounds present in the antagonists by Sherlock MIS software. The antagonist GNRAJK1 was identified as *Streptomyces violaceusniger* - *violaceusniger* and GNRAVR14 as *Streptomyces exfoliatus* based on the equivalent chain length for bacterial fatty acids. This present study provides a theoritical and practical explanation of an antagonist explored for control of stem rot caused by *S. rolfsii*.

Key words: *Streptomyces violaceusniger*, *Streptomyces exfoliates*, *Sclerotium rolfsii*, Groundnut, stemrot, biological control, FAME analysis

1. INTRODUCTION

Groundnut is an important oilseed and ancillary food crop of the world. Besides expulsion of oil, it is used for production of groundnut-butter and a host of other food products. India has the largest groundnut growing area in the world and is the second largest producer after China. In India, groundnut is cultivated during Kharif, Rabi and summer seasons under various cropping systems, and it occupies the third place among oilseed crops and grown in an area of about 5.06 m ha with the production of 8.05 mt and productivity of 1583 kg/ha. Among the soil-borne fungal diseases of groundnut, stem rot caused by *S. rolfsii* is a potential threat to groundnut production and is of considerable economic significance for groundnut grown under irrigated conditions. This disease causes severe damage during any stage of crop growth, and yield losses over 25per cent have been reported by [1]. Control of *Sclerotium* diseases is difficult and needs combination of different methods like cultural, biological and chemical methods for management. Though there are so many biocontrol agents in commercial use. An attempt was made to explore a new organism for the disease management. The genus *Streptomyces* was well known as antifungal biocontrol agents that inhibit several plant pathogenic fungi [2]; [3]; [4]. In the present study the *Streptomyces* species was identified by a step by step analysis.

2. **RESEARCH METHODOLOGY**

Morphological and biochemical identification of bacterial isolates

Characterization of the different cultures of antagonistic bacteria was done according to the methods recommended in the laboratory guide for identification of plant bacteria published by the American Phytopathological Society (Schaad, 1992). For each test, 24 to 48 h old cultures were used.

Methods of analyzing cell wall amino acid (Becker *et al .,* 1965)

Specific method (Thin layer chromotagraphy) for analysing the chemical character of actinomycetes is given as flow charts and is developed by IMTECH, Chandigarh. Different characteristics determined were based on the standard works of cell wall (Becker *et al*., 1965)

Hydrolyses 3 mg of dry cell with 1ml of 6N HCL

↓ (100 degree over night)

Cool and filter add 1ml of H_2o

↓

Dry and evaporate

↓

Dissolve in 1ml of H_2o

↓

Dry and redissolve in 0.3 ml H_2o

↓

Use silica cellulose TLC plate

↓

Load 3ml of sample 1ml of 0.01M (L-diaminophimelic acid)

↓

Develop using-methanol: pyridine: glacial acetic acid : H_2o(5:0.5:125:2.5 v/v)

↓

Spray 0.4% ninhydrin-heat 105 degree for 5 min

↓

Bands appear in light brown

Identification of selected antagonists by light microscopy

The antagonist was observed under the light microscope in order to confirm the genus based on morphology and spore structure. The observation was made using 400 X magnification.

Biolog analysis

The antagonists groups were reconfirmed with Biolog analysis. Identification of microorganism using fully automatic OMNILOG micro biological identification system utilizing yeast micro titre plate.

Identification of *Streptomyces* species by Fatty Acids Methyl Esters (FAME) Analysis

The Sherlock MIS Software was used with the Agilent technologies 5890, 6890 or 6850 gas chromatographs. The Sherlock System's unique configuration was designed for optimal analysis of Fatty Acid Methyl Esters by gas chromatography.

3. FINDINGS AND DISCUSSION

The rhizosphere soils from different locations were collected to screen out the promising antagonist against *S. rolfsii*. Among the hundred isolates tested for their antagonistic activity by dual culture technique, the two isolates *viz*., (Groundnut Rhizosphere Antagonist from Vriddhachalam) GNRAVR14 and (Groundnut Rhizosphere Antagonist from Jayankondam) GNRAJK1were found to be effective. Among the isolates, GNRAJK1 showed highest per cent mycelial growth inhibition of 85.00 per cent followed by GNRAVR14 and GNRAJK3 having 81.25 and 78.75 per cent respectively. The promising rhizosphere antagonists *viz*., GNRAVR14 and GNRAJK1 were taken for further studies. These two unknown isolated antagonists were further subjected for identification.

Bio chemical characterization of bacterial antagonists

The most effective unknown rhizosphere antagonists *viz*., GNRAVR14 and GNRAJK1were taken for bio chemical studies to identify the group of microorganism. The bio chemical studies *viz*., Gram staining, Motility test, Indole test, Methyl Red test, Voges Proskauer test, Citrate test and Catalase test were carried out. The results revealed that the above two antagonists showed positive reaction in gram staining and citrate test (Table 1) Which are the most important characters of the Group Streptomycetes.

The medium specific growth studies also revealed that the above two isolates were grown luxuriantly in the yeast extract malt agar specific for Actinomycetes. In the nutrient broth, growth of the culture was restricted to the top of the broth. It formed pellicle and cottony sediment leaving the broth quite clear which is specific character of Streptomycetes.

Table 1.Biochemical characterization of *Streptomyces* isolate

S.No.	Name of the test	Culture GNRAJK1	Culture GNRAVR1
1	Gram staining	+ve	+ve
2	Motility	Non motile	Non motile
3	Reaction to Indole	-	-
4	Reaction to methyl red	-	-
5	Reaction to Voges Proskauer	-	-
6	Utilization of citrate	+	+
7	Production of Catalase	-	-

Thin Layer Chromatography

The cell wall aminoacids of the above two antagonists were studied by Thin Layer Chromatography and the results revealed that, the strain posses LL-DAP (diaminopimelic acid) along with glycine indicating the cell wall type -1 which are prevalent in the group of *Streptomyces*, *Streptoverticillium*, *Actinopycnidium*, *Actinosporangium*, *Elytrospora*, *Microellobosporia*, *Sporicthya*. (Plate 1)

Plate 1. Detection of LL-DAP (diaminopimelic acid)

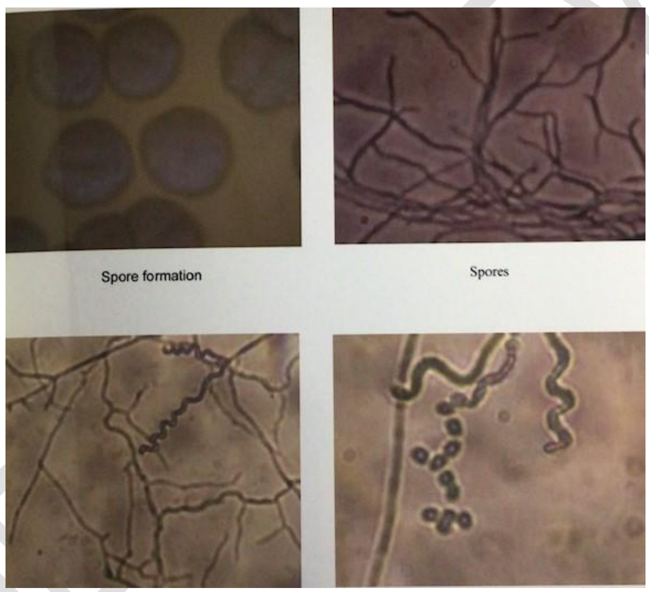
Identification of the selected antagonists by light microscopy

The selected antagonists were observed under the light microscope in order to confirm the genus based on morphology and spore structure. The observation of thin strands of mycelium with spiral spore chains, the predominant character of *Streptomyces* at 400 X magnification again confirmed the genus.

Biolog analysis

The antagonists groups were reconfirmed with Biolog analysis. The above two selected antagonists were confirmed as *Streptomyces* by studying the following characters *viz*., colony type (rough), tiny shaped colony, growth condition (35-39°C), Period of growth (3days in starch casein agar, 4 days in actinomycetes isolation agar, 2 and half days in oat meal agar) and morphological characters (Coil shaped spore forming at 400X magnification) (Plate 2)

Plate.2. Morphology of Streptomyces Sp



Identification of *Streptomyces* species

The antagonists groups were further reconfirmed by identifying 9 and 20 length carbon fatty acids and related compounds present in the antagonists by Sherlock MIS software used with Agilent technologies 5890, 6890, or 6850 gas chromatography. Retention time data was obtained from injecting the calibration mixture was converted to equivalent chain length (ECL) data for bacterial fatty acid naming. Based on the ECL derivation 0.006 and SIM Index of 0.007 the antagonist GNRAJK1 was identified as *Streptomyces violaceusniger* - *violaceusniger* (Fig1& 2) and the closely related species were *Streptomyces lavendulae* and *Streptomyces halstedii- olivaceus*. The antagonist GNRAVR14 was identified as *Streptomyces exfoliatus* with ECL derivation of 0.003 and SIM Index of 0.013 and the closely related species were *Streptoverticillum-septatum* and *Streptomyces lavendulae*. This results corroborated with the findings of [5; 6,7] . *Streptomyces* species exhibit biocontrol activity and it correlates with their production of antibiotics [8]. *Streptomyces violaceusniger* isolated from turf grass rhizosphere and it inhibited seven soil borne pathogens *viz*., *Fusarium oxysporum*, *Pythium ultimum*, *Pythium aphanidermatum*, *Gaeumannomyces graminis*, *Colletotrichum graminicola*, *Rhizoctonia solani*, *Microdochium nivale* and *Sclerotinia homeocarpa*[7]. *Streptomyces hygroscopicus* isolated from the rhizosphere of orchid and showed its *in vitro* antagonism against *Colletotrichum gloeosporioides* and *Sclerotium rolfsii* [9]. Though there was no report on *S. exfoliatus* which produce polysaccharide degrading enzyme have practical applications in fields. Some groups of bacteria are more amenable to fatty acid composition analysis for identification than others. This result corroborated with the findings of [10, 11, 12, 13] enumerated that *Streptomyces* species exhibit biocontrol activity and it correlates with their production of antibiotics.

4. CONCLUSION

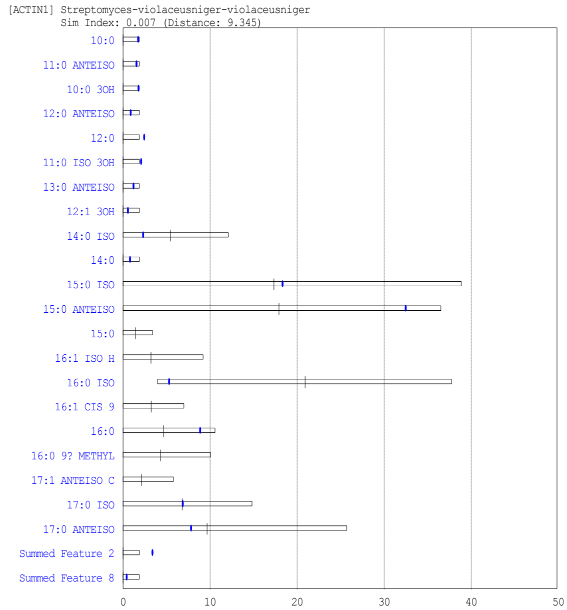
This study provides a theoritical and practical explanation of an antagonist explored for control of ground nut stem rot caused by *S. rolfsii*.

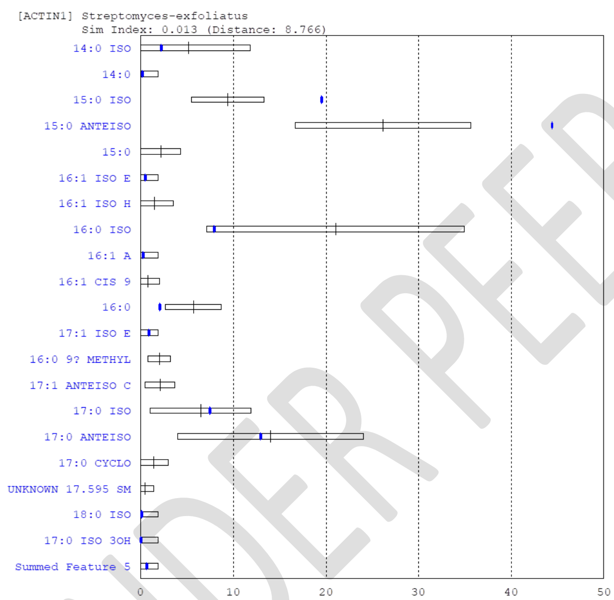
COMPETING INTERESTS

Authors have declared that no competing interests exist.

Fig.1. Dendrogram

Fig.2. Sherlock Report





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