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Research Article

BIOLOGICAL CONTROL OF SCLEROTIUM BY *P. FLUORESCENS* ISOLATED FROM FOREST SOILS

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ABSTRACT
Sclerotium rolfsii is a soil borne pathogen infects number of plant species. In the present study *Pseudomonas fluorescens* strains have been isolated from forest litter of Seshachalam hill range and their antagonistic activities were assessed *in vitro*. All antagonistic isolates produced an inhibition zone varied from 2.0cm to 4.5cm and radial growth inhibition percentage from 43.75% to 73.75%. However isolates PSTPT18 and PSTPT19, were found to be potential antagonists against *Sclerotium* with almost 73.75% of radial growth inhibition**.**

Key Words: *Sclerotium, Poly phagus, Pseudomonas florescence, Biological Control*

INTRODUCTION
Sclerotium rolfsii is a polyphagous soil born pathogen infecting over 500 plant species worldwide causing huge losses. Though the fungus is seed and soil borne, Soil borne inoculum is more important in causing infection and disease development. For the soil borne pathogens, use of fungicides is not practical due to exorbitant cost and environmental hazards involved. Hence integrated management of the disease using biocontrol agents and chemicals is the best. The pathogen is distributed in tropical and subtropical regions of the world where high temperature prevails. The fungus has a wide host range of 500 species in about 100 families including vegetables, flowers, cereals, forage plants and weeds. Some of the common hosts include Legumes, Crucifers, Tomato, Chrysanthemum, Peanuts and Tobacco in which the pathogen causes a great economic loss. In ground nut, it caused 25 % of seedling mortality in the cultivar JL- 24 at parbhani (Ingale and Mayee 1986). Thiribhuvanamal *et al.,* (1999) observed that 30 % of crop loss in tomato was due to *S. rolfsii.* Harinath Naidu (2000) reported that *S. rolfsii* caused 40.05% mortality in *Crossandra* in Chittoor district of Andhra Pradesh.
Several chemical pesticides are used to mange this disease (Bharadwaj *et al.,* 1983; Dutta and Das, 2002 and Bhat and Srivastava, 2003). Indiscriminate use of chemical pesticides in modern agriculture has resulted in the development of several problems such as pesticide resistance in pest resurgence of target and non- target pests, destruction of beneficial organisms like honey bees and chemical residues in food, feed and fodder. More over fungicidal application as seed or soil treatment however has been found to be ineffective against these pathogens as the propagules are capriciously distributed in the soil and often beyond the reach of chemicals (Campbell, 1989). Biological control therefore holds a promise as a strategy for disease management and it is environment friendly too. Antagonistic bacteria especially P.fluroscence have been widely used against a number of phyto pathogens (Bell *et al.,* 1982 and Rini and Sulochana, 2006). For successful functioning of introduced microbial bio inoculants and their influence on soil health efforts have been made to explore soil microbial diversity of indigenous community their distribution and behavior in soil habitats (Hill, 2010).

MATERIALS AND METHODS
Soil samples were collected from forest soils of Tirumala hills, Andhra Pradesh. Randomized block design was employed to collect the samples. Collected soils were sealed in sterile polyethylene bags. For isolation of Bacteria one gram soil from different sampling sites was placed in 9 ml of saline solution and incubated for 2 hours in an orbital shaking incubator at 180 rpm. Later a loop of the resulting bacterial

suspension was spread plated on King's B Agar medium (King *et al.*, 1954) and incubated at 37^0C. After 2 days the colonies were screened for fluorescence under UV light (366nm).

A total of 10 *P. fluorescens* strains were obtained from forest soils and were screened for biocontrol activity against *Sclerotium rolfsii*. Antagonism of bacteria against *Sclerotium* was examined using a modified method of Montealerge *et al*., (2003) a loopful of culture from each purified bacterial isolate was inoculated in to 50 ml of King's B agar broth and incubated for 48 hours at 37^0C. Subsequently, 100µl of bacterial suspension of each isolate was placed on different 10 mm diameter sterile paper discs (Whatman, UK) four different discs were spaced around a central 10 mm plug of 2 day old *Sclerotium* on potato dextrose agar. The plate was incubated for 7 days at 30 ^{0}C and the size of the inhibition zone of hyphal growth was determined. Bacteria which showed no suppression of fungal growth were discarded. The inhibition test was replicated three times. The active bacterial isolates were preserved in 20% glycerol at -20^0C.

Four bacterial isolates showed antagonistic activity in the pre evaluation test were subjected to further confirmation. By the standard co- inoculation PDA technique (Sakthival and Manickam, 1987). The bacterial plugs (6mm diameter) were removed from a 48 hrs grown culture on king's B plate. The bacterial plugs were transferred to the surface of PDA plates, which had been inoculated with fungal spore suspension (or) mycelial plug. After the plates were incubated at 28 ^{0}C for 3 days radial growth percentage of the test fungi was measured using following formula

$$RI\ (\%) = Rc\text{-}Ri/Rc\ *100$$

Where

RI=Radial growth inhibition
Rc= Radial growth in control plates
Ri= Radial growth in incubated plates

In order to authenticate the isolates Phenotypic characters like gram's reaction, levan production, optimum growth temperature, fluorescence, Gelatin hydrolysis, Citrate utilization test, Oxidase, β-galactosidase activity, Catalase test, Indole production were conducted for the bacterial isolates.

Substrate utilization profiles were also tested using Hi carbohydrates (Hi media, Mumbai, India). Cell suspension was established in sterile saline using 24 hours grown culture. The density of the suspension was made to 0.5 O.D at 620 nm. An aliquot of 50µl of this suspension was inoculated at 30^0C for 48 hrs and on to the substrates such as lactose, xylose, fructose, galactose, glycerol, trehalose, manifold, sucrose, ribose, glucose and incubated at 30^0C for 48 hours with the intention to know the inhibition mechanism HCN production test was conducted by using filter paper pre soaked in picric acid solution (Wei et al., 1981). The production of HCN was assessed by taking the following criteria:

No color change	No HCN production	
Brownish coloration	Weak HCN Production	Weak HCN production
Brownish to Orange	Moderate HCN production	Moderate HCN production
Complete Orange	Strong HCN production	Strong HCN production

RESULTS

A total of 10 strains of *P. fluorescens* were isolated from forest soils. Most of the isolated bacteria developed pale green to dark green pigmentation on king's B agar and released a sweet grape like odour and pyocyanine pigment. This was an indication that isolated bacteria were pseudomonads. By exposing the plates to UV light fluorescent pseudomonads were picked up. Among them a total of 4 isolates were found to inhibit mycelial growth on PDA plates in a triplicate assay.

Table 1: Biocontrol activity of *Pseudomonas* with *Sclerocium rolfsii*

Isolate	Distance of fungal migration in cm	Radial growth inhibition
1	4.5	43.75
2	3.5	56.25
3	2.5	68.75
4	4	50
5	3.5	56.25
6	3.7	53.75
7	5	50
8	3	62.5
9	2.1	73.75
10	2.3	71.25

All antagonistic isolates produced an inhibition zone varied from 2.0cm to 4.5cm (Figure 1) and radial growth inhibition percentage from 43.75% to 73.75%.However isolates PSTPT18 and PSTPT19, were found to be potential antagonists against *Sclerotium* with almost 73.75% of radial growth inhibition (Table-1) percentage.

Table 2: Morphological and biochemical characteristics of *P. fluorescens* isolates

S. No	CHARACTER	PSTPT1	PSTPT2	PSTPT18	PSTPT19
1	Fluorescence	Positive	Positive	Positive	Positive
2	Grams reaction	Negative	Negative	Negative	Negative
3	Levan Production	Negative	Negative	Positive	Positive
4	Motility	Positive	Positive	Positive	Positive
5	Optimum growth temp(^{o}C)	4^{o}C	4^{o}C	4^{o}C	4^{o}C
6	Gelatin hydrolysis	Positive	Positive	Positive	Positive
7	Citrate utilization	Positive	Positive	Positive	Positive
8	Oxidase	Positive	Positive	Positive	Positive
9	ONPG	Negative	Negative	Negative	Negative
10	Arginine Hydrolysis	Positive	Positive	Positive	Positive

Table 3: Carbohydrate Utilization by *P. fluorescens* isolates

S. No	CARBOHYDRATE	PSTPT1	PSTPT2	PSTPT18	PSTPT19
1	Lactose	Acid	Acid	Acid	Acid
2	Xylose	Acid	Acid	Gas	Acid
3	Fructose	Gas	Acid	Gas	Acid
4	Galactose	Gas	Gas	Gas	Gas
5	Glycerol	Gas	Acid	Acid	Acid
6	Trehalose	Gas	Gas	Gas	Gas
7	Mannitol	None	None	Gas	Acid
8	Sucrose	Acid	Acid	Acid	Gas
9	Ribose	Gas	Acid	Gas	Acid
10	Glucose	Acid	Acid	Gas	Gas

Figure 1: HCN production by the isolate PSTPT 19. A- Plate showing HCN production by the isolate PSTPT 19

All the 4 isolates showed positive results for Fluorescence, Oxidase, arginine hydrolysis and Gelatin hydrolysis. However, two strains PSTPT18and PSTPT19 were able to produce levan. All the isolates were negative for ONPG production and showed optimum growth at 4^{o}C (Table-2).
All the 4 strains were able to utilize Lactose, xylose Fructose, Glactose, Glycerol, Trehalose, sucrose, Ribose and Glucose and produced either acid or gas (Table-3). However, PSTPT1 and PSTPT2 were unable to utilize mannitol. All the strains were able to produce HCN. However the isolate PSTPT 19 was strong HCN producer which turned the colour of the filter paper in to complete orange. The remaining three Isolates were moderate HCN (Figure 1).

DISCUSSION

Soil is considered as a store house of microbial activity .These functions of soil microorganisms is central to the decomposition process and nutrient cycling. They play an important role in soil processes that determine plant productivity. Pseudomonas sp., belonging to plant growth promoting *Rhizobacteria* has received prominent attention because of the dual role of these bacteria in plant growth promotion and diseases control. Cook (1993) reported that certain plant associated bacteria particularly P.flourescence has been exploited for suppression of crop diseases. Our work demonstrates the ability of *P. fluorescence* to produce fungistatic metabolites such as HCN. Pseudomonas sp. are known to produce volatile compounds. One such metabolite is HCN (Tripathi and Johri, 2002). Afsharmanesh *et al.*, (2010) suggested that fungal growth is mainly inhibited by HCN production and siderophore production Similar results on the effectiveness of fluorescent pseudomonads against plant pathogenic fungi like *Fusarium, Rhizoctonia, Sclerotium, Pythium* (Rao *et al.,* 1999; Ganeshan and Manickam, 1987; Rini and Sulochana, 2007; Leon *et al.,* 2009; Asha *et al.,* 2011; Rokni *et al.,* 2008; Al – Hinai *et al.,*2010) and bacteria like *Ralstonia solanacearum* and *Xanthomonas campestris* have been reported earlier .The effectiveness of fluorescent pseudomonads against multiple pathogens are also known (Muralidharan, 2004).
A part from the biocontrol potential, *P. flourescence* possess other functional properties like, mineral phosphate solubilisation, production of plant growth promoting substances and enzyme activity. Results also revealed that the antifungal activities and other plant beneficial traits appear to be the general and genetically dispersed traits of *P. fluorescens*. Knowledge on phenotypic and biochemical traits of bacteria will help to determine their fitness for successful biofertilization and biological control. This study provides essential information to develop broad spectrum biocontrol agent against Sclerotium.

REFERENCES

Ingale RV and Mayee CD (1986). Efficacy and Economics of some management practices of fungal diseases of ground nut. *Journal of Oil Seeds Research* **3** 201-204.

Thiribhuvanamala G, Rajeswari E and Sabitha Doraiswamy (1999). Inoculum levels of *Sclerotium rolfsii* on the incidence of stem rot in tomato. *Madras Agricultural Journal* **86** 334.

Harinath Naidu (2000). Crossandra- a new host record for *Sclerotium rolfsii. Indian Phytopathology* **53** 496-497.

Bharadwaj CL, Shyam KR and Singh BM (1983). Evaluation of fungicide for the control of seedling blight of rice caused by *Sclerotium rolfsii*. *Indian Journal of Mycology And Plant Pathology* **13** 256-261.

Pranab Dutta and Das BC (2002). Management of collar rot of tomato by *Trichoderma sp*. and chemicals. *Indian Phytopathology* **55** 235-237.

Narayana Bhat and Srivastava LS (2003). Evaluation of some fungicides and neem formulations against six soil borne pathogens and three Trichoderma sp. in *Citrus. Plant Disease Research* **18** 56-60.

Campbell R (1989). Biological control of microbial plant pathogens. *Cambridge University Press, Cambridge* 432.

Bell DK, Wells HD and Markham CR (1982). Invitro antagonism of Trichoderma spp. against six fungal plant pathogens. *Phytopathology* **72** 3.

Rini CR ansd Sulochana KK (2006). Management of seedlings rot of chilli (Capsicum annumm L.) using Trichoderma spp. and fluorescent Pseudomonads (*Pseudomonas fluorescence*). *Phytopathology* **72** 379-382.

Hill GT (2000). Methods for assessing the composition and diversity of soil microbial communities. *Applied Soil Microbiology* **15** 25-36.

King EO, Ward MN and Raney DE (1954). Two simple media for the demonstration of pyocyanin and fluorescein. *Journal of Laboratory and Clinical Medicine* **44** 301-307.

Montealegre JR, Reyes R, Perez LM, Herrerea R, Silva P and Besoain X (2003). Selection of bioantagonistic bacteria to be used in biological control of *Rhizoctonia solani* in Tomato. *Electronic Journal of Biotechnology* **6**(2) 115-127.

Sakthival N and Gnana Manickam SS (1987). Evaluation of Pseudomonas fluorescens for suppression of sheath rot disease and enhancement of grain yields in rice (Oryza sativa L.). *Applied and Environmental Microbiology* 2056-2059.

Wei G, Kloepper JW and Tuzum S (1981). Induction of systemic resistance of cucumber to *Colletotrichum orbiculare* by selected strains of plant promoting rhizobacteria. *Phytopathology* **81**1508-1512.

Cook RJ (1993). Making greater use of Introduced microorganisms for biological control or plant pathogen. *Annual Review of Phytopathology* **31** 53-58.

Tripathi M and Johri BN (2002). *In vitro* antagonistic potential of fluorescent pseudomonas and control of sheath blight of maize caused by *Rhizoctonia solani*. *Indian Journal of Microbiology* **42** 207-214.

Castric KF and Castric PA (1983). Method for rapid detection of cyanogenic bacteria. *Applied and Environmental Microbiology* **45** 701-702.

Rao VS, Sachan IP and John BN (1999). Influence of fluorescent pseudomonas on growth and nodulation of lentil (Lens esculentus) in Fusarium infested soil. *Indian Journal of Microbiology* 39 23-29.

Ganeshan P and Gnana manickam SS (1987). Fungal antagonistic bacteria in rhizosphere soil. *Biology and Biochemistry* **19** 35.

Rini CR and Sulochana KK (2007). Management of seedlings rot of chilli (Capsicum annumm L.) using Trichoderma spp. and fluorescent Pseudomonads (*Pseudomonas fluorescence*). *Phytopathology* **72** 379-382.

Leon M, Yaryura PM, Montecchia MS, Hernandez AI, Correa OS, Pucheu NL, Kerber NL and Garcia AF (2009). Antifungal activity of selected Indigenous *Pseudomonas* and *Bacillus* from the soybean Rhizosphere. *International Journal of Microbiology* 1-9.

Asha BB, Chandra Nayaka S, Udaya Shankar AC, Srinivas C and Niranjana SR (2011). Biological control of F.oxysporium f.sp. lycopersici causing wilt of tomato by Pseudomonas. *Fluorescence* **3**(2) 79-84.

Rokni Zadeh H, Khavazi K, Asghar zadeh A, Hosseini M and Demot R (2008). Biocontrol of Pseudomonas sevastanoi, Causative agent of Olive knot disease Antagonistic potential of Non – pathogenic Rhizosphere Ioslates of Fluorescent Pseudomonas Comm. Appl. Biol. Sci. Ghent University **73**(1) 199-203.

Al – Hinai AH, Alsadi – AM, Al- Bahry SN, Mothershaw AS, AL- Said FA, AL- Harthi SA, and Deadman ML (2010). Isolation and characterization of *Pseudomonas aeruginosa* with antagonistic activity against Pythium aphanidermateus. *Journal of Plant Pathology* **92**(3) 653-660.

Muralidharan K, Reddy CS, Krishnadevi D and Laha GS (2004). Field application of fluorescent pseudomonas products to control blast and sheath blight diseases in rice. *Journal of Mycology and Plant Pathology* **34** 411-414.