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Fluorescent Pseudomonads for plant disease control

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Some of the fluorescent pseudomonads have been reported to show inhibitory properties against some plant pathogens. Some such bacteria were isolated from soil. These are characteristically short rods, gram-negative, non-capsulated and non-spore forming and showed good growth on both solid and broth media like KBC, PDA, Potato peel and less growth on NA. The tests for production of catalase and oxidase enzymes produced by the bacteria were positive. They also showed positive arginine dehydrolase reaction and did not produce H_2S and acetoin. The bacteria showed faculty of reducing nitrate. They also showed alkaline reaction on milk. Test for MR, VP and hypersensitive reactions were positive. The bacteria were good inhibitors of plant pathogens. Percent growth inhibition against *A. niger* (52.38 & 50.74), *F. oxysporum* f. sp. *ciceri* (37.50 & 34.37), *F. oxysporum* f. sp. *udum* (40.0 & 51.42), *Macrophomina phaseolina* (41.81 & 43.63), *Drechslera oryzae* (58.53 & 67.07) *Alternaria solani* (43.10 & 57.72) by the application of the two bacterial isolates were observed. Inhibition zones (diameter in mm) formed by the culture filtrate of the two isolates against *A. niger* and *D. oryzae* came in the range of 25.5 to 9 37 by fish-spine technique when 24 to 48 hrs old cultures were used. An overall 75% to 62.5% disease reduction had been shown in case of Hog-plum fruit rot due to *Aspergillus niger* by the treatment of the fruits with culture filtrate and cell suspension of the bacteria. Reduction of brown spot disease of rice due to *Drechslera oryzae* upto 39.84% and 28.46% had been observed when rice plants were treated with cell suspension before inoculation.

Key words : Fluorescent Pseudomonads, Biological control, Brown spot, Fruit rot

Disease of crop plants are responsible for a good amount of loss in production. Starting from breeding of disease resistant varieties different methods have been engaged in different occasions for minimising this production loss. Biological control of plant disease by using microorganisms, although known for long time, has received a thrust in recent times to be used in practical field. Many micro-

organisms have been identified in this regard (Klement *et al*, 1966). Green fluorescent bacteria also come under group of control agents (Teliz-Ortiz *et al*, 1960, Unnamalai *et al*, 1984, Mehair *et al*, 1981). In the present work *Pseudomonas fluorescens*, a commonly occuring bacteria in soil isolated from soil has been tried for the purpose of controlling two different types of diseases namely brown spot of rice (*Oryza sativa* L.) and fruit rot of Hog-plum (*Spondias mombin* Linn.). Initial selection of the isolates of the bacteria by testing their inhibitory properties have been made *in vitro*.

MATERIALS AND METHODS

Isolation of fluorescent pseudomonads was done from vegetable fields under the Department of Plant Pathology, B.C.K.V. Initially the bacteria were isolated in King's medium B (King *et al*, 1954) Soils diluted in sterilised water upto 10^{-4} to 10^{-5} dilution were plated in King's medium. Petriplates were incubated at 40°±1°C in B.O.D. incubator for growth of fluorescent bacteria. Cultures of bacteria were purified by suspending them in sterilized distilled water and restreaking The cultures were finally maintained in KBC slants at 5°C for further use. Only two isolates named as culture No I and culture No. II were capable of producing yellowish green fluorescent in KBC media selected on the basis of inhibition tests on some fungal plant pathogens. Characterisation of the two isolates of the bacteria was done following the methods described by Kiraly *et al*, (1974).

Inhibitory properties of the isolated bacterial cultures were studied by different methods. In cross-streaking technique, PDA slants were inoculated with test fungi and were streaked face to face with 48 hrs old bacterial cultures. Slants were then incubated at 35°±1°C in B.O D. incubator. Each treatment (T) was replicated five times with equal number of control treatments (C). After 6 days of incubation, fungal growth inhibition was measured by following formula,

$$\text{Percent growth inhibition} = \frac{C-T}{C} \times 100.$$

Where, C = growth in control tubes (mm)
T = growth in treatment tubes (mm)

In Fish spine technique, 24 to 48 hrs old bacterial broth cultures were centrifused at 5000 rpm to get cell free culture filtrate. A portion of the decanted culture filtrate was sterilised for 15 minutes at 15 lb/sq inch in an autoclave. Another portion of centrifused culture filtrate was used for inhibition test as unsterilised one. Spore suspension (5×10^5/ml) of *Aspergillus niger* and *Drechslera oryzae* was sprayed on the surface of PDA plates by an atomiser under aseptic conditions. Sterilised fish spines were dipped into each category of

bacterial culture filtrate and put on the petriplates aseptically. Petriplates were then incubated at 28°±1°C. Radial inhibition was measured after 6 days. The trial on control of fruit rot of hog-plum (*Spondias mombin*) due to *Aspergillus niger* was conducted in laboratory, taking mature green fruits of 2.0×1.0 cm size. Fruits were inoculated with spore suspension of the pathogen (5×10^5/ml) by spraying after smearing of carborandum powder on the fruit surface. The fruits were treated with cell suspension and culture filtrate of fluorescent bacteria (3×10^7 cells/ml) 24 hrs prior to and after inoculation. Untreated but inoculated and uninoculated control were maintained keeping five replications for each. Fruits were incubated under bell-jar at room temperature (18-30 C). The extent of fruit rot was estimated visually in terms of percent surface area infected and rotten.

The trials on control of brown spot of rice (*Oryza sativa*) was also conducted in laboratory. Spore suspension ($5x10^5$/ml) prepared from 7 day old PDA culture of *D. oryzae* was used for inoculation by spraying. Bacterial cell (48 hrs old) suspensions ($3x10^7$/ml) were sprayed on Dular variety of rice plants grown in pots (10' dia.) by using hand atomiser as pre and post inoculation treatments at 24 hrs. interval. Disease assessment has been made following the methods of Sinha and Das (1972). Four different types of spots based on the relative size, namely large, medium, small and minute, were counted from different leaves of the plants. Each type of spot has been assigned a value of according to its size as follows, such as large spot 1.0, medium spot-0.5, small spot-0.25 and minute spot 0.01. The spots developed on leaves were counted and then number of each group was multiplied by the respective assigned value. Sum total of such values gave the disease index of a plant as sum of the disease index recorded from all plants divided by number of plants counted gave the mean disease index for a plant in a treatment. The effect of treatment was expressed as percent inhibition of symptom expression (disease index) in terms of that in control plants.

RESULTS AND DISCUSSION

Isolation and characterisation of green fluorescent bacteria

Isolation of fluorescent bacteria have been made from soils of a vegetable field under the Dept. of Plant Pathology in large number by soil dilution method and 27 isolates of green fluorescent in nature have been detected on King's medium B. On the basis of another selection study following cross-streaking principle in slants only two best inhibitors of at least 7 pathogens have been selected (Table 1). The data on the inhibitory property of the selected isolates toward 7 plant pathogens clearly indicates satisfactory inhibition of *D. oryzae* and *A. niger*. Culture II showed good inhibition of *Fusarium oxysporum* f. sp *udum* and *A. solani* in addition.

Table 1. Growth inhibition of different fungi by fluorescent *Pseudomonas*.

Fungi	Per cent growth inhibition*	
	Culture No. I	Culture No. II
Aspergillus niger	52.38**	50.74
Fusarium oxysporum f. sp. *ciceri*	37.50	4.37
Fusarium oxysporum f. sp. *udum*	40.00	51.42
Macrophomina phaseolina	41 81	43.63
Drechslera oryzae	58.53	67.07
Alternaria solani	43 10	57.72
Sclerotium rolfsii	0	0

* Growth inhibition by cross-streaking. ** Average of 5 replications.

Table 2. Growth inhibition of *Aspergillus niger* and *Drechslera oryzae* with culture filtrates of fluorescent *Pseudomonas*.

Age of culture filtrate (hrs)		Diameter of inhibition * zone (mm)			
		Culture No. I		Culture No. II	
		A niger	*D. oryzae*	*A. niger*	*D. oryzae*
24	NS	13.18**	18.15	13 74	25.5
	S	10.62	12.83	12.37	22.35
	C	0	0	0	0
36	NS	11.25	17.75	12.46	25.15
	S	10.53	13.75	11.55	19.95
	C	0	0	0	0
48	NS	11 03	16 65	11.35	3 05
	S	9.37	11.53	10.75	19.15
	C	0	0	0	0

* Growth inhibition by fish spine technique. ** Average of 15 replications. NS=non sterilizad culture filtrate. S=sterilized culture filtrate C=Control

Table 3. Control of fruit rot of hog-plum by fluorescent *Pseudomonas*.

Treatments		Extent* of fruit rotting by *A. niger*	
		Pre inoculation treatment	Post inoculation treatment
Culture No I	Cell suspension	25**	50
	Culture filtrate	50	75
Culture No. II	Cell suspension	0	50
	Culture filtrate	25	75
Inoculated control		100	100
Uninoculated control		0	0

* Percent of fruit rotting. ** Average of 5 replications.

Table 4. Control or brown spot disease of rice with fluorescent *Pseudomonas*.

Treatment	% reduction of brown spot disease	
	Pre-inoculation	Post-insculatisn
Culture No. I (cell suspension)	28.46*	0
Culture No. II (cell suspension)	39.84	0
Untreated Control	0	0

* Each treatment has an average of 5 replications, each replication contains 10 plants/ each pot.

The isolates were studied following standard determinative procedures (Kiraly *et al*, 1974) and were found to be gram-negative aerobic, rod shaped bacterium, motile possibly due to presence of flagella. The cultures I and II produced green fluorescent pigment in an iron free medium (King's medium B) at a normal growth temperature of 38°-40°C. The bacterium was tentatively identified as *Ps. fluorescens* after being compared with a standard isolate previously confirmed from CMI.

In another test for the inhibitory property of the cultures, culture filtrates when applied through fish-spine in PDA plates seeded with the bacterium following methods of Monda' and Mukherjee (1978) and inhibition zones formed are presented in Table 2. These data also confirmed the inhibitory property of the cultures both under sterilized and unsterilized conditions. Here also culture II appeared to be a better inhibitor.

Control of fruit rot of hog-plum and brown spot of rice

As were the indications from *in vitro* test results, good inhibition of fruit rot has been obtained through application of fluorescent bacterium and better results have been obtained with cell suspension when applied as pre-inoculation treatment. (Table 3). A prior protective spray with cell suspension could protect the injured, inoculated hog-plum fruits against *Aspergillus niger* quite satisfactorily.

In case of the foliar disease i.e. brown spot of rice, however, the reduction has not been satisfactory even when the cell suspension was applied prior to inoculation spray. Post inoculation treatment has been a total failure. It seems that two possible reasons may be assigned for this failure. First of all the bacterium being a natural inhabitant of soil has been successful in harbouring an aerial plant surface to combat the pathogen. As a matter of short coming in the experiment, the inoculation spray might have dislodged the bacterial cells physically from the leaf surface. However the reasons of the bacterium being put in an uncommon habitat for this particular isolate may be the actual reasons behind the failure. The success of using green fluorescent bacterium as a biocontrol agent for

checking a storage disease is significant and deserves extensive trial on different other storage pathogens.

REFERENCES

King, E. O., Ward, M. K. and Raney, D. E., (1954). Two simple media for the demonstration of Pyocyanin and fluoresccin. *J lab. Clin. Med.* **44**, 301-307.

Kiraly, Z., Klement, Z., Solymosy, F. and Voros. J., (1974). *Methods in Plant Pathology.* Akademia Kiado, Budapest.

Klement, Z., Kiraly, Z., Pozsar, B. I. (1966). Suppression of virus multiplication and local lesion production in tobacco following interaction with a saprophytic bacterium. *Acta phytopath. Acad. Sci. Hung.*, **1**, (1/2). 11-18.

Mehair, F. F., Hevesi M., Klement Z., (1981). Components of *Ps. fluorescens* causing the early and late induced resistance of tobacco to challenge infection. *Acta. Phytopath. Acad. Sci. Hung.*, **16**, (3/4), 365-369.

Mondal, R., and Mukherjee, N., (1978). Sensitivity of plant bacteria to some antibiotics, antibacterials and other drugs. *Zeitschrift fur pflanzenkrankheitan and pflanzenschutz.*, **85**, (10), 607-616.

Sinha, A. K. and Das, N. C., (1972). Induced resistance in rice plants against *Helminthosporium* infection. *Physiol. Pl. Path.*, **2**, 401-410.

Singh, Narendra, and Singh, R.S.,(1980). Inhibition of *Fusarium oxysporum* f. sp *udum* by soil bacteria. *Indian Phytopath.* **33** (2), 356-359.

Teliz-Ortiz,M., and Burkholder, W.H.,(1960). A strain of *Pseudomonas fluorescens* antagonistic to *Ps. phaseolicola* and other bacterial plant pathogens. *Phytopathology.* **50**, 119-123.

Unnamalai, N., Gnanamanicham, S. S., (1984) *Pseudomonas fluorescens* is an antagonist to *Xanthomonas citri* (Hasse) Dye, the incitant of citrus canker. *Current Science India.* **53** (13), 703-704.

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