J. Mycopathol. Res. 30(1) : 65-70 (1992) *Printed in India*

Antagonistic bacteria against fungal pathogens

K. K. HAJRA, D. C. KHATUA AND N. MUKHERJEE
Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, 741252

A number of bacteria isolated from different surfaces viz. soil, water, plant etc., each dominating one surface were tested for their antagonistic property against five fungal plant pathogens. Four isolates showed antagonistic property against two pathogens. *Aeromonas* sp. and a *Pseudomonas* sp. were antagonistic to *Macrophomina phaseolina*, a damping-off pathogen. Two isolates of *Pseudomonas*, *Aeromonas* and *Sporosarcina* inhibited the growth of *Penicillium digitatum*, a fruit rot pathogen. All the antagonistic bacteria produce heat stable antifungal compound(s).

Key words : Antagonistic bacteria, *Pseudomonas* sp., *Aeromonas* sp. *Sporosarcina* sp., *Macrophomina phaseolina*, *Penicillium digitatum*

In recent years, to avoid the harmful effects of chemical pesticides on agroecosystem and environment, biological control of plant pathogens through the use of antagonists is being considered as a promising aspect of disease management (Cook and Baker, 1983). Bacteria occupy an important position as potential antagonists. Some of the bacterial agents are proved efficient in controlling damping-off disease through seed bacterisation (Brown, 1974).

M. phaseolina, a soil borne plant pathogen, having a wide host range including jute, potato, chilli, tomato, etc. is reported to be controlled through seed bacterisation by some actinomycetes (Merriman, 1974 ; Mukherjee, 1985 ; Sing and Mehrotra, 1980) and *P. aeruginose* (Ghosh, 1988). *Bacillus subtillis* has been reported to control post harvest fruit rot of citrus by *Alternaria citri* (Farooqui *et al.*, 1981 ; Vapindar and Deverall, 1984) and *Geotrichun candidum* (Vapindar and Deverall, 1984). The present programme includes to explore the

presence of antagonistic bacteria against some soil brone plant pathogens including *M. phaseolina* and *P. digitatum* causing damping-off and fruit rot diseases respectively.

MATERIALS AND METHODS

Both the pathogenic microorganisms and their inhibitor or antagonistic microorganisms occur in nature on different surfaces and substrates. In order to get some cultures of such antagonistic bacteria, considering that antagonists are present on different surfaces, serial isolations were made from the different surfaces of different catagories like plants, soil, water, root surface and laboratory basin (Table 1). The surfaces concerned were washed under aseptic conditions with sterilized distilled water taken in definite volume and such washing was plated on PDA following dilution plating technique. In case of water, it was directly poured in PDA. The colonies grown in highest frequencies after 48-72 hrs. incubation at 30°±1°C were considered as target colonies. One such colony was picked up and streaked on PDA slants followed by purification. Thus ten bacterial isolates were obtained from ten different surfaces (Table 1). The antagonistic property of such bacterial isolates was tested by cross streaking (Ghosh, 1988) against five fungal plant pathogens namely *M. phaseolina, Rhizoctonia oryzae-sativa, Fusarium oxysporum* f. sp. *ciceri, P. digitatum* and *Aspergillus niger*. Each bacterial isolates was inoculated separately at the base of the culture tube and then a pathogenic fungus was inoculated very close to it. The inoculated slants were incubated at 30° ±1°C for 3 days. Necessary control slants were maintained and the observation on the extent of inhibition (Ray *et al.*, 1990) of the fungal pathogen, if any, was recorded (Table 1).

RESULTS AND DISCUSSION

From the inhibition study by cross streaking (Table 1) it was evident that four bacterial isolates viz. LA1, LA 2, TS 1 and TP 1 had antagonistic property against *P. digitatum* and LA 2 gave highest per cent inhibition (85.2%). Only two bacterial isolates namely LA 2 and TS 1 were antagonistic to *M. phaseolina* and TS 1 inhibited growth upto 80 per cent. The bacterial isolates did not have any effect on *R. oryzae-sativa, F. oxysporum* f. sp. *ciceri* and *A. niger*.

In vitro *sensitivity of* M. phaseolina *and* P. digitatum *to culture filtrate of the antagonistic bacteria.*

On the basis of inhibition, two test fungi namely *M. phaseolina* and *P. digitatum* were selected for *in vitro* sensitivity to culture filtrate of the antagonistic

Table 1. *In vitro* inhibition of five fungal plant pathogens by bacteria isolated from ten different surfaces

Bacterial isolates	Source	Percent growth inhibition of plant pathogenic fungi				
		M.p.	R.o.s.	F.c.c.	P.d.	A.n.
S 1	Soil	0.0	0.0	0.0	0.0	0.0
W 1	Lab. tap water	0.0	0.0	0.0	0.0	0.0
RM 1	Rhizosphere of mustard	0.0	0.0	0.0	0.0	0.0
TP 1	Potato tuber	0.0	0.0	0.0	69.8	0.0
TS 1	Rotten Sankhalu	80.0	0.0	0.0	72.9	0.0
G 1	Ginger rhizome	0.0	0.0	0.0	0.0	0.0
FP 1	Pineapple fruit	0.0	0.0	0.0	0.0	0.0
LM 1	Mustard leaf	0.0	0.0	0.0	0.0	0.0
LA 1	Wash basin	0.0	0.0	0.0	64.2	0.0
LA 2	Used lab. glass wares	60.0	0.0	0.0	85.2	0.0

M.p. = *Macrophomina phaseolina*, R.o.s. = *Rhizoctonia oryzae-sativae*,
F.o.c. = *Fusarium oxysporum* f. sp. *ciceri*, P.d. = *Penicillium digitatum*,
A.n. = *Aspergillus niger*

bacteria. Eight day old culture of *M. phaseolina* was blended with sterilized water and strained to prepare a sclerotial suspension. A conidial suspension of *A. niger* was prepared by washing the surface of the sporulating culture. The sclerotial and conidial suspensions were separately mixed with melted PDA (42°C) and poured in sterile petridishes and allowed to solidify.

Four bacterial isolates namely LA 1, LA 2, TS 1 and TP 1 were separately grown in PD broth for hours. The cell free culture filtrates were prepared by centrifuging at 3000 rpm for 15 minutes. The culture filtrate of each isolate was divided into two parts—one part was sterilized by passing through sintered glass filter and the other part by autoclaving for 15 minutes at 121°C. The culture filtrates so prepared were used as such or diluted by adding same volume (1:1) of sterilized distilled water. Sterilized fish spines were dipped into each type of culture filtrate and three such fish spines were placed on the solidified PDA (Mondal and Mukherjee, 1978). The plates were incubated at 30° ± 1°C for 48 hours to note tho inhibition of fungal growth around the fish spines and result is presented in Table 2.

Both original and diluted culture filtrates (50%) sterilized through sintered glass filter of LA 2 and TS 1 inhibited growth of *P. digitatum* and *M. phaseolina*. The inhibitory property of culture filtrate was retained even after autoclaving. The culture filtrate of LA 1 and TP 1 did not show any inhibition on *M. phaseolina* but on *P. digitatum* even after autoclaving. This indicates that all these bacteria produce heat stable antifungal compound(s) in culture medium.

Table 2 *In vitro* sensitivity of *M. phaseolina* and *P. digitatum* to culture filtrate of four antagonistic bacteria

Bacterial isolates	Autoclaved culture filtrate		Unautoclaved culture filtrate	
	50% dilution	Original	50% dilution	Original
		M. phaseolina		
LA 1	—	—	—	—
LA 2	+	++	++	+++
TS 1	+	++	++	+++
TP 1	—	—	—	—
		P. digitatum		
LA 1	+	++	++	+++
LA 2	+	++	++	+++
TS 1	+	++	++	+++
TP 1	+	++	++	+++

— = No inhibition
\+ = Degree of inhibition

Identity of the antagonistic bacteria

To determine the identity of the antagonistic bacteria a series of standard bacteriological studies were conducted (Shekhawat, 1972) on cell morphological and biochemical characteristics and the data are presented in Table 3.

Isolates LA 1 and LA 2 were Gram-ve, rod shaped, aerobic, non-capsulated, non-spore former bacterium producing oxidase, lipase, H_2S, NH_3 and were unable to produce tyrosinase or agrinine dihydrolase or liquefy gelatin. The isolates might be the members of the genus *Pseudomonas*. The third antagonistic bacterium, TS 1, was Gram-Ve, rod shaped, non-capsulted, non-spore former, motile, facultatively anaerobic and produced characteristic non fluorescent red pigment. The bacterium was identified as *Aeromonas*. Another bacterium, TP 1 showing antagonistic property was spherical, Gram+ve, capsulated, spore former, aerobic but non-motile in nature. The speres were found generally singly like *Micrococcus* and occasionally in tetrads. The isolate was identified as *Sporosareina*.

Fluorescent *Pseudomonas* is known as fungal antagonist (Ray *et al.*, 1990) but the present isolates LA 1 and LA 2 isolated from laboratory were non-fluorescent type. The isolates differ in their antagonistic property, one inhibiting the growth of *P. digitatum* and the other inhibiting both *P. digitatum* and *M. phaseolina*. *Aeromonas* and *Sporosarcia* are not repoted earlier as fungal antagonists. Production of heat stable antifungal compound in culture medium by four bacteria deserves further testing of such property *in vivo*.

Table 3. Characteristics of antagonistic bacteria

Characteristics	Isolates of antagonistic bacteria			
	LA 1	LA 2	TS 1	TP 1
Shape	Rod	Rod	Rod	Spherical
Size (μm)	1.0-1.5x0.5-0.75	1.5-2x0.5-0.75	2-2.5x1-1.5	0.4-0.6
Gram reaction	—	—	—	+
Capsule formation	—	—	—	+
Spore production	—	—	—	+
Motility	+	+	+	—
Pigment production	N. Fl. Yellow	No pigment	N. Fl. Red	N. Fl. P nk
Respiratory mechanism	Aerobic	Aerobic	Facultatively anaerobic	Aerobic
Starch hydrolysis	—	+(partial)	—	—
Galatin liquefaction	—	—	Slow	Slow
Catalase production	+	—	—	+
Oxidase production	+	+	Delayed positive	+
Lipase production	+	+	+	+
Tyrosinase production	—	—	+(low)	—
Arginine dihydrolase production	—	—	—	—
H_2S production	+	+	+	—
Acetion production	—	—	—	—
Levan production	—	—	—	—
NH_3 production	+	+	+	+
Milk utilization	Acid curdling and clearing	Rennate curdling	Acid curdling	Acid curdling
Methyl red test	+	+	—	—

N. Fl. = Non fluorescent

REFERENCES

Brown, M. E. (1974). Seed and Root bacterisation. *Ann. Rev. Phytopathol*, **12**, 181-197.

Cook, R. J. and Baker, K. R. (1983). *The Nature and Practice of Biological Control of Plant Pathogens*. The American Phytopathological Society, St. Paul, Minnesota.

Farooqui, W.A., Ahamad, M., Khanum, S.A., and Hatton, T.T. (1983). Studies on the shelf-life extension of citrus fruits. In proceedings of the International Society of citriculture, 1981, Vol : 2, Shimizu, Japan, Fruit Tree Research Station (1983, 762-761).

Ghosh, T. K. (1988). Suppressive bacteria for control of soil-borne plant diseases. M. Sc. (Ag.). Thesis. B.C.K.V., West Bengal, India.

Merriman, P. R., Price, R.D., and Baker, K.F. (1974). The effect of inoculation of seed with antagonists of *Rhizoctonia solani* on the growth of wheat. *Aust. J. Agric. Res.* **25**(2), 213-218.

Mondal, R. and Mukherjee, N. (1978). Sensitivity of plant bacteria to some antibiotics, antibacterials and drugs. *Z. Pflan. Pflanzenchutz*, **85**(10), 607-616.

Mukherjee, B. (1985). Some effects of Ecol. behaviour of *Macrophomina phaseolina* (Tassi) Goid. Ph. D. Thesis, B.C.K.V., West Bengal India.

Ray, S., Ghosh, M. and Mukherjee, N. (1990). Fluorescent Pseudomonads for plant disease control. *J. Mycopathol Res* **28**(2), 135-140.

Shekhawat, G. S. (1972). Plant Bacteriology, Vol. II, Phytopathological Techniques.

Singh, R. S. and Mehrotra, R. S. (1980). Biological control of *Rhizoctonia bataticola* by coating seeds with *Bacillus subtilis* and *Streptomyces* and their influence in plant growth. *Plant and Soil*, 56, 475-483.

Vapinder, Singh and Deveral, B. J. (1984). *Bacillus subtilis* as control agent against fungal plant pathogen of citrus fruit. *Trans. Brit. Mycol. Soc.* **83**(3), 487-490.

(Accepted for pulication 14 November 1991)