

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Variations Between Strains of *Pseudomonas* Bacterium I: Effects on Root-Infecting Fungi

Nasima Imam Ali, Imran Ali Siddiqui, M. Javed Zaki and ¹Maqsood Ali Ansari
Department of Botany, University of Karachi, Karachi-75270, Pakistan
¹Department of Genetics, University of Karachi, Karachi-75270, Pakistan

Abstract: Twenty isolates of *Pseudomonas* sp., were tested for the control of soilborne root-infecting fungi like *Macrophomina phaseolina*, *Fusarium solani* and *Rhizoctonia solani* under laboratory and greenhouse conditions. In dual culture plate assay, strain 78 inhibited all the test fungi. Other *Pseudomonas* sp., failed to check the radial growth of *M. phaseolina* whereas isolates 51 and 82 significantly inhibited radial growth of *F. solani* and *R. solani*. Three isolates of *Pseudomonas* sp., (51, 78 and 82) when used as seed dressing or as soil drench showed substantial reduction in root-rot infection caused by *M. phaseolina*, *F. solani* and *R. solani* in mungbean. Strain 78 was found to be most effective in the control of root-infecting fungi. Of the root-infecting fungi, *R. solani* was most susceptible to *Pseudomonas* sp.

Key words: Biological control, *Pseudomonas* spp., root-rot

Introduction

Several soilborne pathogens cause root-rot and root-knot diseases in mungbean [*Vigna radiata* (L.) Wilczek] and may seriously limit production (Siddiqui *et al.*, 1999). Of these, *Fusarium solani* (Mart.) Appal and Wollenw. Emend Snyder and Hans., *Macrophomina phaseolina* (Tarsi) Gold, *Rhizoctonia solani* Kuhn are reported to cause serious problems in mungbean production (Ehteshamul-Haque and Ghaffar, 1994). Since many crops have little or no resistance to many diseases, use of microorganisms in the biological control of disease is an alternate method of plant disease control (Lumsden and Lewis, 1989).

Research on biological control agents has emphasized free-living, plant-associated, non-pathogenic bacteria present in the rhizosphere and rhizoplane. Of the various rhizosphere bacteria, Fluorescent pseudomonads (*Pseudomonas fluorescens*, *P. putida*, *P. aeruginosa* and some closely related species) are common inhabitants of the rhizosphere and contribute to the control of many soilborne plant diseases caused by fungi (Alabouvette *et al.*, 1993; Buchenauer, 1998). In this paper we discuss some experiments on the effectiveness of *Pseudomonas* bacteria in the control of root-infecting fungi such as *Macrophomina phaseolina*, *Fusarium solani* and *Rhizoctonia solani*.

Materials and Methods

Microorganisms used: Twenty strains/isolates of *Pseudomonas* sp., were obtained from the culture collection of the Department of Genetics, University of Karachi. The bacteria were maintained on King's B medium for 5 days at 25 ± 2°C and used in the present study. Root-infecting fungi viz., *M. phaseolina*, *F. solani* and *R. solani* were isolated from the infected roots of mungbean and maintained on potato dextrose agar (PDA) at 25 ± 2°C for one week.

In vitro studies: Using technique as suggested by Drapeau *et al.* (1973), the strains/isolates of bacteria were streaked on one side of the Petri dish containing Czapek's Dox Agar medium, pH 7.2 whereas on the other side a 5-mm diam., disc of root-infecting test fungus was inoculated. The dishes were incubated at 25 ± 2°C and zone of inhibition (if any) was recorded after one week.

Greenhouse studies: Of the twenty isolates of

Pseudomonas sp., used in the present study, only three showed promising results in the inhibition of radial growth of root-infecting fungi and were selected for their use as seed dressing and soil drench in the control of root-infecting fungi on mungbean under greenhouse conditions. Soil used in the present study was a sandy-loam (sand:silt: clay, 70:19:11), pH 8.1 with moisture holding capacity of 40% obtained from the experimental field of the Department of Botany, University of Karachi. The soil had a natural population of 4 to 11 sclerotia g⁻¹ of soil of *M. phaseolina* as assessed by wet sieving and dilution technique (Sheikh and Ghaffar, 1975); 7% colonization of *R. solani* on sorghum seeds used as baits (Wilhelm, 1955) and 2800 CFU g⁻¹ of soil of *Fusarium solani* as assessed by soil dilution technique (Nash and Snyder, 1962). The soil was transferred in 8-cm diam., plastic pots at 350 g per pot.

For seed dressing, mungbean seeds after surface sterilization in 1% Ca(OCl)₂ for 3 mins., were rinsed several times with tap water and treated with aqueous cell suspension of the bacteria using 1% gum arabic as sticker giving a population of 2.0-2.4 × 10⁶ CFU per seed. After seed treatment eight seeds were sown in each pot and after germination only four seedlings were maintained per pot. Seed treated with sterile distilled water served as controls. For soil treatment, after removing the soil to a depth of 2.5 cm a 25 ml aqueous cell suspension containing 3.0 × 10⁶ CFU ml⁻¹ was drenched in each pot. Eight mungbean seeds were then sown in each pot and surface was covered with soil. After germination four seedlings were kept in each pot. Soil drenched with sterile distilled water was used as a control. There were three replicates of each treatment and pots were kept randomized on greenhouse bench of the Soilborne Diseases Research Laboratory, Department of Botany, University of Karachi. The temperature of the upper soil surface ranged between 25-30°C throughout the experiments.

The plants were uprooted carefully after 45 days growth. To determine the incidence of root-infecting fungi, 1 cm long root pieces were surface sterilized with 1% Ca(OCl)₂ and transferred onto PDA plates supplemented with penicillin (100, 000 units/l.) and streptomycin sulfate (0.2 g/l.) 5 pieces/plate. The plates were incubated at room temperature and after one week infection on roots by fungi

was recorded using the following formula:

$$\text{Infection (\%)} = \frac{\text{No. of root pieces infected by a pathogen}}{\text{Total number of root pieces}} \times 100$$

Results

In dual culture plate assay, isolates of *Pseudomonas* showed variable activity against different root-infecting fungi. Strain 78 of *Pseudomonas* sp., inhibited all the test fungi producing a zone of 8 and 5-mm respectively against *M. phaseolina* and *F. solani* whereas colonies of bacteria and *R. solani* met each other with no further growth recorded of either organism. Similarly, strain 82 produced a zone of 16 and 10 mm against *F. solani* and *R. solani* respectively, whereas strain 51 produced a 20 and 10 mm zone of inhibition respectively, against *F. solani* and *R. solani*. Most of the bacterial isolates used in this study were failed to inhibit *M. phaseolina* (Table 1).

Table 1: *In vitro* inhibition of *Macrophomina phaseolina*, *Fusarium solani* and *Rhizoctonia solani* by different strains/isolates of *Pseudomonas* spp.

Bacterial strains	*Type of reaction zone of inhibition (Film)		
	<i>Macrophomina phaseolina</i>	<i>Fusarium solani</i>	<i>Rhizoctonia solani</i>
<i>P. aeruginosa</i> (58)	B	0	8
<i>P. aeruginosa</i> (71)	B	B	A
<i>P. aeruginosa</i> (27)	B	A	C
<i>P. aeruginosa</i> (74)	B	B	A
<i>P. aeruginosa</i> (82)	B	16	10
<i>Pseudomonas</i> sp. (51)	B	20	10
<i>P. aeruginosa</i> (78)	8	5	A
<i>Pseudomonas</i> sp. (14)	B	13	A
<i>P. aeruginosa</i> (68)	B	A	2
<i>P. aeruginosa</i> (17)	B	7	A
<i>P. aeruginosa</i> (24)	B	B	B
<i>P. aeruginosa</i> (20)	B	B	B
<i>P. aeruginosa</i> (87)	B	B	B
<i>Pseudomonas</i> sp. (68)	B	B	B
<i>P. aeruginosa</i> (34)	B	B	B
<i>Pseudomonas</i> sp. (77)	B	B	B
<i>Pseudomonas</i> sp. (75)	B	B	B
<i>P. aeruginosa</i> (11)	B	B	B
<i>Pseudomonas</i> sp. (80)	B	B	B
<i>P. aeruginosa</i> (35)	B	B	B*

* A = Colonies of both organisms met each other with no further growth of either organism observed.
 B = Colonies of the pathogen overgrew on the bacterium.
 C = A zone of inhibition was initially produced but later on fungus overgrew on the bacterial growth.

Under greenhouse conditions, *Pseudomonas* isolates used as seed dressing or as soil drench although reduced root infections caused by *M. phaseolina* and *F. solani* but their effects were statistically non-significant. Strain 78 of *Pseudomonas* sp., used as seed dressing (>46 %) or as soil drench (>39%) showed greatest reduction in *F. solani* infection. *R. solani* infection was significantly (p<0.05) suppressed following seed dressing with *Pseudomonas* sp., with no significant effects when used as soil drench. Strain 51 and 78 used as seed dressing (>75%) or as soil drench (>66%) resulted in the maximum reduction in *R. solani* infection (Fig. 1).

Discussion

In dual culture plate assay *Pseudomonas* sp., inhibited radial growth of root-infecting fungi viz., *M. phaseolina*,

F. solani and *R. solani* producing zones of inhibition. It interesting to note that different strains of *Pseudomonas* showed variability against different root-infecting fungi. Presumably like rhizobia (Chao, 1990), the antagonist ability of *P. aeruginosa* also varies with the strains used.

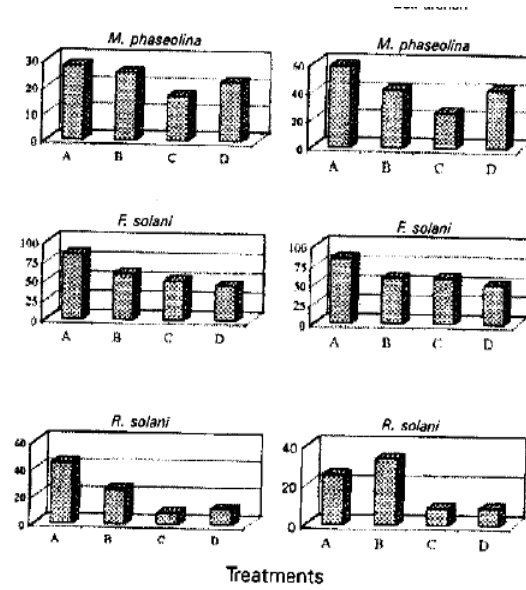


Fig. 1: Effect of seed dressing or soil drench of different strains/isolates of *Pseudomonas* bacteria in the control of *Macrophomina phaseolina*, *Fusarium solani* and *Rhizoctonia solani* in mungbean
 A = Control, B = Strain 82, C = Strain 51, D = Strain 78

In the present study, *Pseudomonas aeruginosa* and *Pseudomonas* sp., either used as seed dressing or as soil drench significantly prevented the infection of *M. phaseolina*, *F. solani* and *R. solani*. The bacteria belonging to the fluorescent *pseudomonas* eg., *Pseudomonas fluorescens* and *P. putida* which colonize roots of a wide range of crop plants are reported to be antagonistic to soilborne plant pathogens like *Rhizoctonia solani*, *Pythium ultimum*, *P. debaryanum* and *P. aphanidermatum* (Suslow and Schroth, 1982). Cultures of *P. fluorescens* used for the seed treatment of wheat showed control of take all disease of wheat (Weller and Cook, 1983). Banana suckers when treated with *P. fluorescens* also reduced the severity of wilt and internal discoloration on banana (Sivamani and Gnanamanickam, 1988). *P. fluorescens* reduced the infection of *Sclerotium rolfsii* on peanut (Ganesan and Gnanamanickam, 1987). Fluorescent *Pseudomonas* has also been reported to reduce *Fusarium* wilt in carnation (Yuen et al., 1985). In the present study, it is interesting to note that *R. solani* was found comparatively susceptible to *Pseudomonas* sp. There are reports where strains of *Pseudomonas fluorescens* and *P. putida* carrying the *chiA* gene coding for chitinolytic enzyme suppressed disease caused by the plant pathogenic fungi *Rhizoctonia solani* and *Sclerotium rolfsii* (Koby et al., 1994). Most of the *Pseudomonas* species are known to survive for a shorter period of time in the rhizosphere after introduction in the soil. Downing and Thomson (2000) in their bean model system demonstrated that *P. fluorescens* Rifl was only required for a brief period

as *R. solani*, the main cause of damping-off disease, rapidly colonizes seedlings and reaches a peak within 2 to 4 days (Sneh *et al.*, 1966). It is therefore suggested that effective biological control using *Pseudomonas* bacterium can be attained in those crop-fungus combinations where yield loss can be prevented by reducing fungal colonization within the first week after germination.

Acknowledgment

The present work was carried out under the research grant of University Grants Commission, Pakistan which is sincerely acknowledged.

References

- Alabouvette, C., P. Lemanceau and C. Steinberg, 1993. Recent advances in the biological control of fusarium wilts. *Pestic. Sci.*, 37: 365-373.
- Buchenauer, H., 1998. Biological control of soil-borne diseases by rhizobacteria. *J. Plant Dis. Prot.*, 105: 329-348.
- Chao, W.L., 1990. Antagonistic activity of *Rhizobium* spp. against beneficial and plant pathogenic fungi. *Lett. Applied Microbiol.*, 10: 213-215.
- Downing, K.J. and J.A. Thomson, 2000. Introduction of the *Serratia marcescens* chiA gene into an endophytic *Pseudomonas fluorescens* for the biocontrol of phytopathogenic fungi. *Can. J. Microbiol.*, 46: 363-369.
- Drapeau, R., J.A. Fortin and C. Gagnon, 1973. Antifungal activity of *Rhizobium*. *Can. J. Bot.*, 51: 681-682.
- Ehteshamul-Haque, S. and A. Ghaffar, 1994. New records of root infecting fungi from Pakistan. *Pak. J. Phytopathol.*, 6: 50-57.
- Ganesan, P. and S.S. Gnanamanickam, 1987. Biological control of *Sclerotium rolfsii* sacc. in peanut by inoculation with *Pseudomonas fluorescens*. *Soil Biol. Biochem.*, 19: 35-38.
- Koby, S., H. Schickler, I. Chet and A.B. Oppenheim, 1994. The chitinase encoding Tn7-based chiA gene endows *Pseudomonas fluorescens* with the capacity to control plant pathogens in soil. *Gene*, 147: 81-83.
- Lumsden, R.D. and J.A. Lewis, 1989. Selection Production, Formulation and Commercial Use of Plant Disease Biocontrol Fungi: Problems and Progress. Cambridge University Press, Cambridge, pp: 171-190.
- Nash, S.M. and W.C. Snyder, 1962. Quantitative estimations by plate counts of propagules of the bean root rot fusarium in field soils. *Phytopathology*, 52: 567-572.
- Sheikh, A.H. and A. Ghaffar, 1975. Population study of the sclerotia of *Macrophomina phaseolina* in cotton fields. *Pak. J. Bot.*, 7: 13-17.
- Siddiqui, I.A., S. Ehteshamul-Haque and A. Ghaffar, 1999. Effects of *Pseudomonas aeruginosa* and chemical fertilizer on root-rot and root-knot disease of mungbean. *Pak. J. Nematol.*, 17: 77-86.
- Sivamani, E. and S.S. Gnanamanickam, 1988. Biological control of *Fusarium oxysporum* f. sp. *cubense* in banana by inoculation with *Pseudomonas fluorescens*. *Plant Soil*, 107: 3-9.
- Sneh, B., J. Katan, Y. Henis and I. Wahl, 1966. Methods for evaluating inoculum density of *Rhizoctonia* in naturally infested soil. *Phytopathology*, 56: 74-78.
- Suslow, T.V. and M.N. Schroth, 1982. Rhizobacteria of sugar beets: Effects of seed application and root colonization on yield. *Phytopathology*, 72: 199-201.
- Weller, D.M. and R.J. Cook, 1983. Suppression of take-all of wheat by seed treatments with *Fluorescent pseudomonads*. *Phytopathology*, 73: 463-469.
- Wilhelm, S., 1955. Longevity of the *Verticillium wilt* fungus in the laboratory and field. *Phytopathology*, 45: 180-181.
- Yuen, G.Y., M.N. Schroth and A.H. McCain, 1985. Reduction of *Fusarium* wilt of carnation with suppressive soils and antagonistic bacteria. *Plant Dis.*, 69: 1071-1075.