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Survival of *Pseudomonas aeruginosa* in various carriers for the inhibition of root rot-root knot disease complex of mungbean

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Summary. The survival of *Pseudomonas aeruginosa* strain 78 was tested on mungbean seeds coated with a variety of substrates/carriers and was found best on talc amended with carboxymethyl cellulose or on gum arabic. *Albizia saman*- and *Cordia myxa*-gum gave poor survival. On all substrates the antagonist populations declined dramatically at 120 days after coating. In the repeating experiments, a seed coating with talc-based inoculum of the antagonist caused marked reduction in nematode population densities in the soil and roots and also reduced subsequent root-knot development due to *Meloidogyne javanica*, the root-knot nematode. However, the incidence of the root-infecting fungi *Macrophomina phaseolina* and *Rhizoctonia solani* did not differ significantly from the controls. Strain 78 significantly promoted growth with increased *Bradyrhizobium*-nodules in mungbean.

Key words: biological control, *Pseudomonas aeruginosa*, root-rot, root-knot, seed treatment.

Introduction

Seed-treatment is an attractive method for introducing biocontrol agents into the soil-root environment. A seed coating with microbial antagonists protects the seed from soilborne pathogens and enables it to germinate and become healthy seedlings (Windels, 1981). The commercial use of biocontrol agents requires inoculum that retains high cell viability and can easily be transported and applied to the seed. In most studies requiring bacterial inocula, either liquid suspensions (Broadbent *et al*., 1977; Burr *et al.*, 1978; Kloepper *et al*., 1980) or bacteria mixed with peat (Roughley and Vincent, 1967; Nair and Fahy, 1976; Davidson and Reuszer, 1978) have been applied. The use of methylcellulose in a powder form to coat plant growth-promoting rhizobacteria (PGPR) on sugarbeet seed showed that PGPR also survive well in dried form under field conditions (Suslow, 1980a, 1980b). In our previous study, strain 78 of *Pseudomonas aeruginosa* (Schroeter) Migula used as seed dressing or as soil drench showed promising results in the suppression of root-infecting fungi including *Macrophomina phaseolina, Fusarium solani* and *Rhizoctonia solani* (Ali *et al*., 2001). The present paper examines: i) the viability of *P. aeruginosa* strain 78 on talc powder-carboxymethyl cellulose (CMC) or various gums of plant origin, and ii) the feasibility of seed treatment with talc-based inoculum to control the root-infecting fungi *Macrophomina phaseolina* (Tassi) Goid., *Fusarium solani* (Mart.) Appel & Wollenw. Emend Snyd. & Hans., and *Rhizoctonia solani* Kühn, and the root-knot nematode *Meloidogyne javanica* (Treub) Chitw., in mungbean [*Vigna radiata* (L.) Wilczek].

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Materials and methods

P. aeruginosa strain 78 was obtained from the Department of Genetics, University of Karachi. The bacterial inoculum was produced by transferring two loopfuls of the bacterium from a 5-day-old culture to 100-ml King's B liquid medium (King *et al*., 1954) and incubating at room temperature on a shaker (150 rpm) for 48 h. The bacterial culture was centrifuged (4,500 *g*, 15 min), the supernatant was discarded and the pellet resuspended in sterile $MgSO_4$ (0.1 M). The ability of *P. aeruginosa* to survive in various carrier materials was tested. The carriers tested included gum arabic, *Albizia saman*-gum, *Cordia myxa*-gum and talc powder-CMC. Twenty-percent w:v (prepared in distilled water) autoclaved gum arabic, *Albizia saman*-gum and *Cordia myxa*-gum were used as sticking substance. Ten ml of each gum was mixed with 10-ml of a $3.1x10^9$ cfu ml^{-1} suspension and placed in a polyethylene bag. Mungbean seeds were added to the bags. The talc-based bacterial inoculum was prepared by mixing 1 g CMC, 1 kg commercially available talc powder and 2.5 l water, adjusting the pH to 7.0 with calcium carbonate and autoclaving at 15 psi for 20 min. One kg of the carrier was inoculated with 400 ml of the bacterial suspension containing $3.1x10^9$ cfu ml^{-1} and kept in sealed polythene bags at room temperature (35±2°C). Mungbean seeds were added to each polyethylene bag and shaken vigorously. After 10 min, the seeds were removed from the bags and checked for the presence of an initial population of the bacterium. Samples were drawn at intervals up to four months after inoculation. At each draw, viable populations of *P. aeruginosa* per seed were determined by transferring ten treated-seeds to a test tube containing 10-ml sterilized distilled water. The test tube was shaken, 10-fold serial dilutions of the suspension were prepared and 0.1-ml aliquots of each were spread on King's B medium. Treatments were replicated three times and Petri dishes were incubated at room temperature. After three days, cfus of the bacterium were recorded. Since talc-CMC yielded populations of *P. aeruginosa* strain 78 greater than those by other carrier materials this substrate was selected for further study.

Soil used for the greenhouse experiment was a sandy loam (sand:silt:clay, 70:19:11), pH 8.1 with a maximum moisture holding capacity of 37%, obtained from an experimental field near the Department of Botany, University of Karachi. This soil was found to be naturally infested with the three root-infecting fungi as follows: 5–9 sclerotia of *M. phaseolina* per g of soil as determined by wet sieving and dilution (Sheikh and Ghaffar, 1978); concentration of *R. solani* producing 6.8% colonization on sorghum grains used as bait (Wilhelm, 1955); and 2,800 cfu g^{-1} of a mixed population of *Fusarium* spp. per g of soil, determined by soil dilution technique described by Nash and Snyder (1962). The soil was placed in 21-cm-diam. earthen pots at 2 kg/pot. The soil in each pot was excavated to a depth of 2.5-cm and mungbean seeds treated with *P. aeruginosa* in the talc-CMC substrate were sown in each pot at 8 seeds/pot. Seeds treated with talc-CMC without the bacterium served as controls. There were three replicates of each treatment, pots were randomized on the greenhouse bench in April 2000 and the experiment was repeated in September 2000. The temperature of the upper soil surface in April-May 2000 was 30–37°C, RH 60–70%; in September-October 2000 it was 28–38°C, RH 70-78%. After germination, four seedlings were retained in each pot. One-week after seedling emergence, the soil in each pot was inoculated with 2000 freshly hatched juveniles of *M. javanica*. The juveniles were suspended in 15-ml water that was poured into three holes made around the seedlings. The pots were fertilized with urea at 0.1 g/kg soil every 15 days. The experiment was terminated 45 days after addition of the nematodes and at that time growth parameters such as plant-height, root-length, fresh weight of shoot and root, and number of *Bradyrhizobium*-nodules/plant were recorded. The galls induced by *M. javanica* on the entire root system were counted with the aid of a low-power microscope (x6). The nematode population density in the soil was determined following the Baermann funnel technique. To determine nematode invasion, a 1 g root sample was thoroughly washed and placed in 0.25% fuchsine-lactic acid. Stained roots were macerated in an electric grinder and slurry was suspended in 100-ml water. *M. javanica* females and juveniles were counted in five sample of 5-ml each. To determine the incidence of the root-infecting fungi, roots from each plant were cut into 5-mm-long pieces, surface sterilized with 1% $Ca(OCl)_2$ and plated on PDA supplemented with penicillin (100,000 units/l) and streptomycin sulfate (0.2 g/l). The plates were incubated at room

temperature for 5 days and incidence of the fungi was calculated as follows:

$$\% \text{ Infection} = \frac{\text{No. of infected plants}}{\text{Total No. of plants}} \times 100$$

Data were subjected to analysis of variance (ANOVA) followed by the LSD or Duncan's multiple range test in accordance with Sokal and Rohlf (1995). Data on the bacterial population per seed was transformed to $\log_{10} x + 1$ to achieve homogeneity of the variances.

Results

The talc-CMC substrate yielded higher *P. aeruginosa* strain 78 populations on mungbean seeds (log cfu 4.42 at 120 days) than did other carriers (Table 1). The lowest population of strain-78 occurred on the substrate with *Cordia myxa*-gum. In general, bacterial populations declined steadily over time. The final bacterial populations were reduced to about three log units when mixed with *Albizia saman* or *Cordia myxa*-gum, and to about two log units with gum arabic or talc substrate.

Seed treatments

Experiment 1 (April-May, 2000)

Treatment of mungbean seeds with a talc-based inoculum of strain 78 significantly ($P<0.001$) reduced nematode population densities in the soil (>37%), nematode invasion of the roots ($P<0.05$; 41%) and consequent root-knot development ($P<0.01$; 35%) compared with the controls (Table 2). *P. aeruginosa* did not significantly reduce root-rot infection but reduced *M. phaseolina* and *R.solani* infections to 39 and 33% lower respectively than those of the controls. The number of *Bradyrhizobium*-nodules per root system was significantly greater ($P<0.05$). Plant-height, shoot-weight and root-length increased significantly ($P<0.05$) after seed application with strain 78 as compared with the controls.

Experiment 2 (September–October, 2000)

In the repeated experiment, *P. aeruginosa* strain 78 significantly reduced ($P<0.05$; 10.9%) the nematode populations in the soil, nematode invasion ($P<0.05$; 25.6%) and the galling rate ($P<0.001$; 50.9%) as compared with the controls (Table 2). Root-rot infection by *M. phaseolina* was significantly lower (51.7%) with *P. aeruginosa* ($P<0.01$) than with the controls, but the bacterium did not have a significant impact on the other two soil fungi. Nodulation was significantly increased ($P<0.001$; 440%) by *P. aeruginosa* treatment and plant-height and root-length were increased by 23.3 and 32.7% respectively ($P<0.05$). Shoot-weight was not significantly influenced and root-weight was significantly greater in the controls ($P<0.05$).

Discussion

In the present study, the bacterial population declined markedly with storage time, the reduction

Table 1. Survival of *Pseudomonas aeruginosa* on mungbean seeds after coating with various carrier materials to which a 10^8 cfu/ml suspension of the bacterium had been mixed. Bacterial concentration per seed is expressed as $\log_{10}$ (x+1) cfu per seed.

Bacterial carrier	Time of incubation (days)				
	0	15	30	60	120
Control	0 e	0 e	0 e	0 d	0 e
Albizia saman gum	5.48 d	4.17 d	3.89 c	3.29 c	2.85 c
Gum arabic	5.73 b	5.02 b	4.55 b	4.01 b	3.86 b
Cordia myxa gum	5.35 c	4.95 bc	3.69 d	3.22 c	2.48 d
Talc substrate[a]	6.02 a	5.68 a	5.26 a	4.80 a	4.42 a

Means followed by the same letter in each column are not significantly different according to Duncan's multiple range test.
[a] Talc powder-carboxymethyl cellulose.

achieved by the talc-based substrate was not sufficient to inhibit the disease for up to two months of storage. Kloepper and Schroth (1981) found that a dried-dust substrate of xanthum gum and gum arabic yielded higher PGPR (strain A1) populations than methylcellulose. A talc-based inoculum of *Pseudomonas fluorescens* applied to chickpea seeds significantly reduced *Fusarium*-wilt incidence (Vidhyasekaran and Muthamilan, 1995). It was also demonstrated that *Pseudomonas aeruginosa* strain IE-6, a plant growth-promoting rhizobacterium, survived on a talc-based substrate for 244 days and that such bacterial inoculum when incorporated into the soil significantly lowered root rot-root knot infection in tomato (Siddiqui and Ehteshamul-Haque, unpublished).

Recently, soil application of a *Pseudomonas* bacterium has yielded a substantial degree of control of root-rot and root-knot diseases in tomato (Siddiqui and Ehteshamul-Haque, 2000a, 2000b). However, application of bacterial antagonists with this type of delivery may prove impractical and prohibitively expensive because of the high amounts of inoculum required for adequate pest control. In the present study, an isolate of *Pseudomonas aeruginosa* applied to mungbean seeds effectively controlled root-knot from *M. javanica* and provided a measure of protection against soilborne root-infecting fungi, including *M. phaseolina*, and *R. solani*. Seed-bacterization of radish and seed tubers of potato with *P. fluorescens* strain WCS374 significantly boosted plant growth in a high-frequency radish-and potato-cropping soil (Geels and Schippers, 1983; Geels *et al*., 1985), whereas *P. putida* strain WCS358 increased potato tuber yield (Bakker *et al*., 1986) and root development (Bakker *et al*., 1987) in potato. The advantages of a seed treatment with rhizobacteria in a biological control system are: a) their saprophytic nutritional status allows large-scale production; b) only small amounts of inoculum are required;

Table 2. Effects of seed treatment with talc powder-carboxymethyl cellulose based inoculum of *Pseudomonas aeruginosa* strain-78 on root-knot and root-rot infection by various fungi, *Bradyrhizobium* nodulation and growth of mungbean in April 2000 and September 2000.

	April 2000			September 2000		
	Control	*P. aeruginosa*	LSD	Control	*P. aeruginosa*	LSD
Root-knot infection						
Galls per root: number	59	37	7	77	51	12
Nematode population in 290 g soil	3390	2000	429	4210	3750	384
Nematode population per g root	96	62	31	117	87	26
Root-rot infection						
M. phaseolina: percentage	23	14	17	58	28	22
F. solani: percentage	25	29	25	44	25	20
R. solani: percentage	62	39	39	83	58	36
Bradyrhizobium-nodulation						
Nodules number[a]	11	18	4	5	27	9
Mungbean growth						
Plant-height (cm)	17.2	20.3	1.8	18.4	22.7	2.4
Shoot-weight (g)	3.8	4.9	0.6	2.7	3.3	0.9
Root-length (cm)	7.3	10.9	1.8	5.8	7.7	1.2
Root-weight (g)	0.8	0.5	0.1	0.9	0.6	0.2

[a] Number of nodules per root system.

c) ease of application; d) does not depend on energy sources for survival; e) systemic spread along the surface of the developing root system; and f) antagonistic activity on the root surface during the economically important phase of early root infection by the nematode (Oostendorp and Sikora, 1989).

It is suggested that talc-based inoculum of *P. aeruginosa* could be supplied to the farmers for seed treatment, or to seed producers so that they can supply treated seeds to farmers. Further study should be directed towards formulating bacterial inocula for the longer term and so as to achieve improved viability during storage. The antagonist population per gram of talc can also be increased by using a higher inoculum density of bacteria for commercial use of the preparation.

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