IMPACT OF BIOCONTROL *PSEUDOMONAS FLUORESCENS* CHA0 AND ITS GENETICALLY MODIFIED (GM) DERIVATIVES ON PENETRATION OF *MELOIDOGYNE JAVANICA* IN MUNGBEAN ROOTS

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Summary. *Pseudomonas fluorescens* strain CHA0 and its genetically modified (GM) derivatives CHA0-Rif/pME3424 and CHA89 caused a significant reduction in penetration rates of *Meloidogyne javanica* juveniles in mungbean roots. With increasing nematode densities, nematode penetration rates increased. At low population densities of *M. javanica* (0, 500, 1000 and 2000 J2/pot), all the three strains of *P. fluorescens* gave significantly reduced nematode penetration rates. At the highest population density of *M. javanica* (4000 J2/pot), CHA0-Rif/pME3424 was also effective in the repeated experiments while wild type CHA0 was effective only in one experiment. CHA89 failed to cause significant reduction in nematode penetration at high nematode densities (2000 and 4000 J2/pot). Whereas strain CHA0/pME3424 consistently reduced shoot weight, strain CHA0 had no such effect but CHA89 enhanced shoot growth. Root weight was not markedly altered by any of the strains.

Introduction of beneficial microorganisms into soil or the rhizosphere has been proposed for biological control of soilborne crop diseases (Weller, 1988; Siddiqui *et al.*, 2001). In certain cases, genetically modified (GM) strains with increased expression of biocontrol traits have been developed to improve biocontrol efficacy (Schnider *et al.*, 1995). *Pseudomonas fluorescens* CHA0-Rif produces several bioactive compounds, including the antimicrobial polyketides 2,4-diacetylphloroglucinol (Phl) and pyoluteorin (Plt), and can protect cucumber against *Pythium ultimum* (Voisard *et al.*, 1994). The GM strain *P. fluorescens* CHA0-Rif(pME3424) overproduces the antimicrobial compounds Phl and Plt and displays enhanced biocontrol activity against *P. ultimum* (Schnider *et al.*, 1995). Phl and Plt inhibit the growth of a broad spectrum of bacteria and fungi (Keel *et al.*, 1992). Similarly, a transposon-induced diacetylphloroglucinol (DAPG)-negative biosynthetic mutant of *P. fluorescens* strain F113 and its complemented derivative with restored DAPG synthesis showed that the ability of strain F113 to produce DAPG was responsible for increased hatch ability and the reduction in juvenile mobility of the potato cyst nematode *Globodera rostochiensis* (Cronin *et al.*, 1997). CHA89 (*gacA*::Ω-Km) is a kanamycine resistant (50 µg/ml) derivative of the strain CHA0 and is deficient in antibiotic production (Schnider *et al.*, 2000). Siderophore-negative mutants suppressed diseases less effectively than did wild-type strains (Kloepper and Schroth, 1981).

The aim of the present investigation was to determine whether an antibiotic over-producing derivative of *P. fluorescens* may enhance biocontrol efficacy of the bacteria against *Meloidogyne javanica*.

MATERIALS AND METHODS

The root-colonizing *Pseudomonas fluorescens* strain CHA0 (Faqua and Greenberg, 1998) and its derivatives, which vary in the production of secondary metabolites were used in this study. For example, *P. fluorescens* CHA0-Rif/pME3424 (Schnider *et al.*, 1995), contains *IncP* plasmid (about seven copy per cell) carrying *rpo*D with its natural promoter tetracycline resistant (125 µg/ml) responsible for the overproduction of the antibiotics 2,4-diacetylphloroglucinol and pyoluteorin. On the other hand, strain CHA89 (*gacA*::Ω-Km) is a kanamycine resistant (50 µg/ml) and is deficient in antibiotic production (Schnider *et al.*, 2000). The bacteria were cultivated at 28 °C for 24 h with shaking (150 rpm) in 250-ml Erlenmeyer flasks containing 100 ml of nutrient broth yeast extract (NBY) medium. The bacterial culture was centrifuged at 2,800 x *g* for 20 min., the supernatant discarded and the pellet resuspended in $MgSO_4$ (0.1 M) prior to use. *Meloidogyne javanica* (Treub) Chitw. was obtained from pure cultures maintained on roots of eggplants (*Solanum melongena* L.). The entire root system was dipped in water and soil was removed gently without detaching egg sacs. Eggs were extracted by vigorous shaking of infested roots in a 1% sodium hypochlorite solution for three minutes. The resulting suspension was then passed through a range of different mesh sieves. The eggs collected on a fine sieve (38 µm) were washed in tap water to remove all traces of sodium hypochlorite before use. Hatched juveniles of *M. javanica* were obtained by placing the eggs in sterile distilled water for 3 days at 28 °C.

The sandy-loam soil (sand:silt:clay, 70:19:11%) of pH 8.1 with moisture holding capacity of 39% was obtained from the experimental field of the Department

of Botany, University of Karachi. It was passed through a 2-mm sieve to discard stones and large organic debris and filled in 8 cm diam. plastic pots at 350 g/pot. The experimental design was a 4 x 5 factorial. The factors included four bacterial applications (CHA0; CHA0-Rif/pME3424; CHA89 and no bacterium) and five nematode densities (0, 500, 1000, 2000 and 4000 J2/pot). The upper one cm soil surface was removed and a cell suspension (3.8 x 10^8 cfu ml^{-1}) prepared in 25 ml $MgSO_4$ (0.1 M) was drenched in a pot. Soil drenched with 25 ml $MgSO_4$ (0.1 M) without the bacterium served as control. Subsequently four mungbean [*Vigna radiata* (L.) Wilczek] seeds were sown in each pot and following germination only one seedling retained per pot. One week after seedling emergence, soil in each pot was inoculated with various population densities of *M. javanica*. The juveniles of less than one-week-old were counted and the required inoculum was introduced in 10 ml water, by pipetting the juveniles into three holes around the roots of the seedling. Each treatment had four replicates and pots were arranged in randomized complete block design. The experiment was terminated 21 days after nematode addition and fresh shoot and root weights recorded. To determine the nematode penetration rate, mungbean roots were rinsed with tap water to remove adhering soil, blotted dry, weighed and boiled in 0.1% lactic acid fuchsin. After homogenization in an electric blender, the juveniles that had penetrated the roots were counted with the aid of a low power microscope (x 6). The experiment was repeated once with the same design. Data were analyzed for significance by a factorial analysis of variance using the software STATISTICA ver. 5.0. A Bartlett's test was performed to test the homogeneity of the variances between repeated experiments. In case of nematode penetration rate the chi-square showed significant differences, therefore, the data of the two experiments were presented separately while for the data of shoot and root growth, the variances were homogeneous and the analyses were performed on pooled data sets.

RESULTS AND DISCUSSION

Soil application with *P. fluorescens* strain CHA0 and its genetically modified derivatives CHA0-Rif/pME3424 and CHA89 significantly ($p<0.05$) reduced nematode penetration rates in mungbean (Table I). With an increase in nematode densities in soil, nematode penetration rate increased. However, regardless of the population densities nematode penetration was significantly lower in the treated plants compared to the controls. At all the nematode densities, antibiotic over-producing strain CHA0-Rif/pME3424 was more effective in the reduction of nematode invasion as compared to the wild type CHA0 or antibiotic deficient CHA89. Strain CHA0-Rif/pME3424 was effective at all the population densities in the repeated experiment. However, CHA0 reduced nematode penetration rates only in the first experiment while failed in the repeated experiment. Whereas CHA89 caused significant ($p<0.05$) reduction in nematode penetration at low population densities of *M. javanica*, at high densities (2000 and 4000 J2/pot), the strain failed to demonstrate significant reduction in nematode penetration.

In general, with an increase in nematode densities, fresh shoot weight of mungbean plants decreased (Table II). Regardless of the bacterial strains, these inhibitory effects were more pronounced at high nematode densities. The antibiotic over-producing strain CHA0/pME3424 consistently reduced ($p<0.05$) fresh shoot weight of mungbean at low nematode densities (500, 1000 and 2000 J2/pot) compared to the controls while the wild type strain CHA0 had no significant impact in this respect. When compared to the controls, the antibiotic deficient strain CHA89 increased fresh shoot weight at high nematode densities. The phytotoxicity of the antibiotic overproducing strain CHA0/pME3090 on sweet corn, cress and tobacco has been previously reported (Maurhofer *et al.*, 1995). Surprisingly, neither the wild type strain CHA0 nor its GM derivatives had any substantial effect on fresh weight of roots.

Although statistically significant, the reduction in nematode invasion following soil application with bacteria was only slight and all roots contained significant num-

Table I. Effect of *Pseudomonas fluorescens* CHA0 and its genetically modified (GM) derivatives pME3424 and CHA89 on penetration of *Meloidogyne javanica* juveniles in mungbean roots.

Juveniles per pot	Control			CHA0			pME3424			CHA89		
	ExpI	ExpII	Mean	ExpI	ExpII	Mean	ExpI	ExpII	Mean	ExpI	ExpII	Mean
0	0	0	0	0	0	0	0	0	0	0	0	0
500	47	58	53	33	38	36	24	31	28	36	44	40
1000	66	73	70	42	50	46	38	45	42	51	53	52
2000	79	87	83	55	63	59	48	56	52	75	93	84
4000	103	112	108	85	109	97	73	80	77	100	116	108

($LSD_{0.05}$): Experiment no.1 — Nematode density = 11 — Bacteria = 8

Experiment no.2 — Nematode density = 9 — Bacteria = 7

Table II. Effect of *Pseudomonas fluorescens* CHA0 and its genetically modified (GM) derivatives pME3424 and CHA89 on shoot and root growth of mungbean.

Juveniles per pot	Shoot weight (g)				Root weight (g)			
	Control	CHA0	PME3424	CHA89	Control	CHA0	PME3424	CHA89
0	1.9	2.0	1.5	2.2	0.57	0.54	0.52	0.60
500	2.1	2.0	1.7	2.5	0.63	0.58	0.49	0.65
1000	2.1	1.8	1.5	2.1	0.59	0.57	0.47	0.57
2000	1.6	1.5	1.2	1.9	0.52	0.49	0.51	0.57
4000	1.3	1.6	1.3	1.7	0.55	0.48	0.49	0.59
$LSD_{0.05}$:	Nematode density = 0.39				Nematode density = 0.18			
	Treatment = 0.29				Treatment = 0.15			

bers of nematodes. Therefore, such biocontrol inoculants tend to provide short-term control and are only useful in reducing the invasion of roots by nematodes that have a single generation in the growing season. The results of this study suggest that in the rhizosphere of mungbean, resident antibiotic-producing pseudomonads may reduce penetration of *M. javanica* and subsequent root-knot infection. It is, however, not known that reduced nematode penetration rates by bacteria are due to the production of toxic nematicidal compounds or owing to enhanced host defense mechanism leading to systemic protection. From a biotechnological viewpoint, such antibiotic producing pseudomonads (e.g., strain CHA0-Rif/pME3424) may be useful to develop a biocontrol strategy against *M. javanica* in the early crop growth stages.

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