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## Infection of *Meloidogyne javanica* with *Pasteuria penetrans*

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**Abstract:** *Pasteuria* spore attachment to root knot juveniles reduced significantly ( $p < 0.001$ ), as dilution factor of bacterial suspension was increased. Isolate UK 1 provided maximum spore attachment to *Meloidogyne* juveniles in all three dilutions of bacterial spore suspension (S, S/2 and S/4) compared to other test isolates and control. Infected juveniles by spores of all bacterial isolates (UK 1, PK 1 and PK 2) significantly ( $p < 0.05$ ) suppressed invasion to tomato roots. There was significant difference ( $p < 0.001$ ) in root invasion by nematodes exposed to the different concentrations of test bacterial isolates. Temperatures i.e. 15 and 35°C exhibited reduction in bacterial spore attachment to the root knot juveniles. Whereas 25 and 30°C favored bacterial infection of nematodes.

**Key words:** *Pasteuria penetrans*, *Meloidogyne* spp., spore attachment, temperature, biocontrol potential.

### Introduction

*Pasteuria penetrans* (Thorne) Sayre and Starr is an obligate, most specific pathogen of phytonematodes and cosmopolitan in distribution. Bacterium is frequently encountered in many climates and environmental conditions, it's prolonged period under dry conditions makes it promising for the control of *Meloidogyne* spp. and other economically important plant parasitic nematodes (Sayer and Starr, 1988; Sturhan, 1988; Dickson *et al.*, 1994; Hewlett *et al.*, 1994). The antagonistic potential of *P. penetrans* is reported to be influenced by different abiotic factors (Hatz and Dickson, 1992; Davies *et al.*, 1991; Verdejo-Lucas, 1992; Ouri, 1997; Casida, 1982). Besides, concentration of *P. penetrans* spores may also be influenced by variability in the susceptibility of host nematodes to particular population (Gowen and Tzortzakakis, 1994). A comparable study has been made between indigenous isolates and UK isolate of *P. penetrans* and observed the effect of bacterial infection to root-knot nematode influenced by bacterial spore concentrations, penetration of bacterial infected nematodes in tomato roots and different temperatures.

### Materials and Methods

**Nematode culture:** *Meloidogyne javanica* (Treub) Chitwood population was collected from Karachi University garden, Karachi, was used in this study. Nematode species was cultured on eggplant (*Solanum melongena* L. cv. black beauty) in steam sterilized sandy loam soil under greenhouse condition. Eggs of *M. javanica* were extracted from roots of eggplant in 1-% sodium hypochlorate (Hussey and Barker, 1973). Eggs were collected on 400-mesh sieve and transferred into 100-ml beaker. Egg suspension were put on sieve lined with tissue paper. Freshly hatched second stage juveniles were obtained after 24-48 hours.

***Pasteuria* isolates:** Three isolates of *Pasteuria penetrans* one from UK, designated UK I (PP3-Personal communication) and two local isolates PK 1 (University Garden, Karachi) and PK 2 (Shah Faisal Colony, Karachi) (Zaki, 2000) were selected for comparative study. All test isolates were multiplied on *M. javanica* growing on tomato (*Lycopersicon esculentum* Mill cv. Roma VF) in a greenhouse. *Pasteuria* spore attached J2 (2-3 days old) were collected by centrifugation attachment method (Hewlett and Dickson, 1994). Two-week-old tomato seedlings inoculated with bacterial spore

attached juveniles @ 1000 J2/pot, 3-4 times at one-week interval. A month after inoculation, root systems were chopped and powdered in laboratory grinder. Root powder containing *Pasteuria* spores was used as stock.

**Effect of bacterial spore concentration on bacterial spore attachment to *M. javanica* juveniles:** Spore suspensions of all test isolates (UK 1, PK 1 and PK 2) were prepared according to the method of Stirling and Wachtel (1980). Powdered root containing *Pasteuria* spores were wet grinded in a mortar and pestle in order to release the bacterial spore from plant root tissues. Resulting slurry was passed through a 25- $\mu$ m sieve to separate root debris. The suspension prepared, containing  $10^5$  spores /ml were designated as stock (S) suspension. Further concentrations were (S/2 and S/4 conc.) were prepared by adding requisite amount of sterile distilled water in stock suspension (S). All three test concentrations (S, S/2 and S/4 conc.) of each test isolate were transferred in plastic cavity block diam. 2.5 cm. @ 3 ml in each. Freshly hatched root knot juveniles (@ 40-60 J2 /cavity block) were taken in each cavity block. Set of replicates containing distilled water served as control. All cavity block were randomized and incubated at  $28 \pm 2^\circ\text{C}$  for 48 hrs. At termination of experiment 20 juveniles per replicate were examined under Nikon compound microscope (x40) for bacterial infestation. Number of bacterial spore per juveniles was estimated.

**Penetration of bacterial infected nematodes in tomato roots:** For nematode invasion assay, freshly hatched *M. javanica* juveniles were exposed to S, S/2 and S/4 concentrations of *Pasteuria* isolate UK 1, PK 1 and PK 2 for 24-48 hours. Bacterial spore attachment to second stage juveniles was confirmed for each concentration of all test bacterial strains in a drop of water with the aid of compound microscope (x40). *Pasteuria* infested juveniles suspension of each concentration (S, S/2 and S/4) of all test isolate were introduced (@150-200 J2/3 ml) in depressions made around two weeks old tomato seedlings growing in disposable paper glass (d. 7 cm) containing 200g sterilized sandy loam soil. Pots receiving un-infested juveniles served as control. Plants were harvested after 10 days of nematode inoculation. Roots were washed to remove adherent soil, blotter dried, weighed and boiled with acid fuchsin stain for 3 minutes (Schindler, 1961). Stained roots were washed to remove excess stain, kept in destain and homogenized. Nematode invaded the tomato roots were observed under stereoscope microscope (x10). Number of root-knot juveniles in  $\text{g}^{-1}$  root was calculated.

**Effect of temperature on *Pasteuria* spore attachment to *Meloidogyne* juveniles:** Bacterial spore suspension of each test isolate was transferred in 2.5 cm diam. plastic cavity block @ 3ml/replicate and 60-80 J2 /0.2 ml suspension of freshly hatched juveniles of root knot nematodes were added in each cavity block. All cavity block were kept in incubators at 15, 20, 25, 30 and 35°C. Set of treatments free of bacterial spore suspension served as control. After 24-hrs interval 20 juveniles per replicate were examined under Nikon compound microscope (x40) in a drop of suspension taken on glass slide (1x3 inch) and count the number of bacterial spores attached to nematode cuticle. Experimental data were subjected to factorial analysis of variance (FANOVA). Mean values were compared according to Duncan's multiple range test (DMRT) (Gomez and Gomez, 1984).

**Results**

**Effect of spore concentrations on *Pasteuria* attachment:** *Pasteuria* spore attachment to *M. javanica* root knot nematode was reduced significantly ( $p < 0.001$ ) with increase in dilution factor. In stock suspension of *Pasteuria* isolates,  $> 21$  spores/J2 were found attached with nematode cuticle in UK isolate compared to PK2 ( $> 13$  spores/J2) and PK1 ( $> 11$  spores/J2). Maximum *Pasteuria* spores were observed encumbered in all three dilutions (S, S/2 and S/4) of UK isolate compared to local *Pasteuria* isolates (PK2 and PK1). *Pasteuria* spore attachment was significantly ( $p < 0.05$ ) different among the isolates and their dilution (Table 1).

**Penetration of bacterial infected nematodes in tomato roots:** Penetration of infected juveniles by all test bacterial isolates (UK 1, PK 1 and PK 2) significantly ( $p < 0.05$ ) suppressed into tomato roots. There was significant difference ( $p < 0.001$ ) in root invasion by nematodes exposed to the different concentrations of test bacterial isolates. Maximum suppression in root invasion was observed with stock suspension of UK  $>$  PK 1 and PK 2 compared to other concentrations i.e. S/2 and S/4 and control. Interaction of test isolates and their concentrations were not different significantly in sense of root-knot nematode invasion on tomato roots (Table 2).

**Effect of different temperature regimes on *Pasteuria* spore attachment to *Meloidogyne* juveniles:** All the temperature levels influenced the *Pasteuria* spore attachment to the root juveniles *in vitro*. Bacterial spore attachment was significantly ( $p < 0.01$ ) different among the *Pasteuria* isolates (UK1, PK1 and PK2). Greater bacterial spore attachment was found in UK isolate compared to local isolates. All the temperature regimes (15, 20, 25, 30 and 35°C) significantly ( $p < 0.001$ ) effect the activity of all bacterial isolates. Extremes of the temperature regimes (15 and 35°C) exhibit reduction in bacterial spore attachment to the root not juveniles whereas intermediary temperature levels (25 and 30°C) were found favorable for bacterial spore attachment. At  $30 \pm 2^\circ\text{C}$  all three isolates were remained active by showing greater adherence of bacterial spores to juvenile's cuticle (Table 3).

**Discussion**

Endospores of *P. penetrans* are non-motile and when a suitable nematode host enters its domain, the endospores attach to the nematode's cuticle (Chen and Dickson, 1998). Number of spores attached per juveniles decreased as dilution factor increased (Table 1). Variable spore infection of root knot nematode was observed with each test bacterial isolate (UK 1, PK 1 and PK 2). One to several hundred spores may attach per nematode, however single endospores is sufficient to infect the nematode host. A juvenile carrying larger number of bacterial endospores become less mobile in rhizosphere and can not invade plant root tissues (Stirling and White, 1982). It is therefore suggested that in this study, nematode invasion was suppressed in treatments, which were receiving nematodes with heavy spore load. Besides *P. penetrans* spore infectivity, spores may be influenced by variability in the susceptibility of host nematodes to particular population (Gowen and Tzortzakakis, 1994). Reduced number of bacterial spores to single nematode by all three bacterial isolate may attributed to their compatibility with nematode host as well as lowering of

Table 1: Effect of *Pasteuria* spore concentration on spore attachment to *Meloidogyne* juveniles

Treatments	No. of spore attached/J2		
	Isolate UK 1	Isolate PK 1	Isolate PK 2
Stock concentration (s)	21.45 ± 7.29	11.52 ± 1.54	13.65 ± 1.57
Concentration S/2	12.61 ± 0.75	5.74 ± 1.77	8.75 ± 1.08
Concentration S/4	7.58 ± 0.69	3.35 ± 0.52	5.45 ± 0.81
Control	0	0	0

Significant level (P), Source: Isolate = 15.69 (\*\*\*) , Dilution = 70.06(\*\*\*), Isolate x Dilution = 2.77(\*), \*,  $P < 0.001$

Table 2: Penetration of *Meloidogyne javanica* infected with *Pasteuria penetrans* to tomato

Treatments	No. of nematodes/groot		
	Isolate UK 1	Isolate PK 1	Isolate PK 2
Stock concentration (s)	112	119	121
Concentration S/2	121	124	145
Concentration S/4	143	156	163
Control	175	175	175

Significant level (P), Source: Isolate = 3.58(\*), Dilution = 42.91(\*\*\*), Isolate x Dilution = 0.87(NS), \*,  $P < 0.05$ ; \*\*\*,  $P < 0.01$ ; NS = Non-significant

Table 3: Effect of temperatures level on *Pasteuria* spore attachment to *Meloidogyne* larvae

Treatment (°C)	No. of spore attached/J2		
	Isolate UK 1	Isolate PK 1	Isolate PK 2
15 ± 2	6.55 ± 1.47	5.45 ± 1.73	4.510 ± 1.52
20 ± 2	7.63 ± 2.34	6.35 ± 1.41	5.420 ± 1.6
25 ± 2	10.15 ± 2.21	7.12 ± 1.16	8.560 ± 1.41
30 ± 2	13.35 ± 3.79	10.71 ± 2.00	11.460 ± 0.77
35 ± 2	7.43 ± 0.77	6.56 ± 1.59	5.121 ± 1.35
Control	0	0	0

Significant level (P), Source: Isolate = 5.53(\*\*), Temperature = 50.12(\*\*\*), Isolate = 5.53(\*\*), Isolate x Temp., = 0.6(NS), \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.01$ ; NS = Non-significant

bacterial spore density in sub-dilutions. When *Pasteuria* spore suspension (Stock = S) of UK 1, PK 1 and PK 2 were subjected to different temperature levels. Results revealed that at 15 and 35°C bacterial spore attachment was reduced ( $p < 0.001$ ) compared to juveniles exposed to bacterial spore suspension at 25 and 30°C. *P. penetrans* is cosmopolitan in distribution, so temperature requirement for infection may vary strain to strain. Endospore attachment to root knot juveniles and other nematodes increased with increase in temperature upto 30°C (Singh and Dhawan, 1990; Stirling *et al.*, 1990). Hatz and Dickson (1992) also reported that number of endospores attached per juvenile decline over 30°C. The influence of temperature on the infectivity of bacterial antagonist is obscure but temperature effect on nematode host may be involved in suppressiveness of bacterial infection. Recent studies suggest that protein on the nematode cuticle and endospore surface influenced by temperature may involve in suppression of bacterial attachment. A carbohydrate-protein mechanism is involved in endospore attachment to *M. incognita* (Davies, 1994; Davies *et al.*, 1992, 1996; Davies and Danks, 1993). In this study variation in spore attachment of different bacterial isolates, may be attributed to difference in the surface composition of nematode species, race, population and heterogeneity of the endospore surface which influence by different concentrations of bacterial spore suspension and temperature levels. The obscure mechanism involved in the variable attachment showed that for the exploitation of *Pasteuria* species as biocontrol of root knot nematode, careful and detail investigations are required.

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