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EFFECT OF RHIZOSPHERIC BACTERIA ON PLANT GROWTH OF WHEAT INFECTED WITH *HETERODERA AVENAE*

by

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Summary. Some rhizospheric bacteria were tested in potted plants to assess their possible mitigatory effect against the cereal cyst nematode, *Heterodera avenae* infecting wheat. *Azotobacter chroococcum*, *Azospirillum lipoferum* and *Pseudomonas* sp. reduced nematode infection to various levels while *Rhizobium ciceri* had no apparent effect. Six out of eight strains of *A. chroococcum* showing different properties like nitrogen fixation, ammonia excretion, indole acetic acid (IAA) production and siderophore production reduced cyst formation from 6 to 60 per cent. Strain 1 (1) and Mac 27 having high siderophore production did not control nematode infection but high temperature resistant mutant HT54, possessing the highest ammonia excretion ability and negligible IAA production, reduced nematode infection by 48 per cent. Results indicated that ammonia but not siderophores or IAA had a role in the control of *H. avenae* infection in wheat. All strains of *A. chroococcum* in general and HT 54 in particular promoted growth of nematode infected wheat plants.

Heterodera avenae is an important pathogen of wheat affecting crop production (Rivoal and Cook, 1993). Various rhizobacteria, especially *Azotobacter chroococcum*, are known to excrete ammonia (Narula *et al.*, 1981), produce siderophores (Page, 1987; Suneja and Lakshminarayana, 1993) or release indole acetic acid (Martinez-Toledo *et al.*, 1988) and are likely to be involved in the biological control of nematodes. This article presents the results on the antagonistic effect of diazotrophic bacteria and their possible mode of action against the cereal cyst nematode, *H. avenae* infecting wheat.

Materials and methods

Soil isolates of *A. chroococcum* Beijerinck [103, 6(2), 1(1), 8003, IS-16] and mutants (Mac 21, Mac 27, HT 54) were grown on Jensen agar slants (Jensen, 1951), *Azospirillum lipoferum* Dobereiner (FS) on malate agar slants, *Pseudomonas* on nutrient agar slants and *Rhizobium ciceri* Nour on yeast extract mannitol agar slants for 3-4 days at 30 °C and then maintained at 4 °C. All of the cultures were obtained from the culture collection of Department of Microbiology, CCS HAU, Hisar. Wheat *Triticum aestivum* L. em. Thell seeds cv. WH 147 immersed in the above bacterial culture suspensions (10^8 ml^{-1}) for 5 min were sown in earthen pots filled with 3 kg steam sterilized sandy loam soil. Immediately after emergence, the seedlings were thinned to one plant per pot. Ten cysts of *H. avenae* Wollenw. were inoculated around the exposed roots after one week, covered with soil and irrigated lightly. Each of the treatments were replicated four times and the pots were arranged in a completely randomised design on benches in a screen house. Plant height and

plant dry weight were recorded 14 weeks after sowing. The soil and root balls were depotted into a pan containing water. The roots were carefully washed. Soil in the pan was processed through 60 mesh sieve and the number of cysts (root + soil) counted.

Results and discussion

Out of the four rhizospheric diazotrophs tested (Table I), *A. chroococcum* (HT 54) showed maximum reduction in nematode infection (48%) followed by *Pseudomonas* (11%) and *Azospirillum* (4%). In contrast, *R. ciceri* was totally ineffective. Although *Pseudomonas* was more effective in mitigating nematode infection than *Azospirillum*, the biomass accumulation by wheat was better when inoculated with *Azospirillum*.

To understand the mechanism of nematode inhibition, various strains of *A. chroococcum* with different biochemical and physiological properties such as nitrogenase activity, ammonia excretion, siderophore and indole acetic acid (IAA) production (Table II) were tested.

Soil isolate 103 is IAA producer while its thermotolerant mutant HT 54 is IAA non-producer. Both these strains reduced nematode infection to 60 and 48 per cent, respectively. Similarly, strain 6(2) also a high IAA producer (Verma, 1997), effectively reduced nematode population density. These results indicate that IAA production is not related to nematode inhibition and supports previous observations (Verma and Bansal, 1996) that IAA had the least effect on hatching of *M. javanica* eggs *in vitro*.

The present studies have shown that nematode infection was controlled to varied extent by all strains of *A. chroococcum* except 1(1) and Mac 27 which are high siderophore-producing strains (Suneja and Lakshminarayana, 1993). On the contrary, strain 6(2) which reduced nematode infection to 44 per cent (Table III) does not produce siderophores. From these results it can be assumed that siderophore production has no direct role in controlling nematode infection. This is understandable, because phytonematodes are obligate parasites and depend on the host plant for their nutritional requirement, including iron. Therefore, iron deprivation in the rhizosphere, as a result of chelation due to the action of siderophore cannot be involved in the biocontrol of nematodes. How-

TABLE I - *Effect of bacterization of wheat with various rhizospheric bacteria on biomass and nematode infection.*

Rhizospheric diazotrophs	Average plant height (cm)	Average plant dry weight (g)	Nematode infection	
			Average no. of cysts	Reduction (%)
Control (Nematode only)	52.9	4.7	148	–
Azotobacter chroococcum (HT 54)	58.0	6.3	77	48.0
Azospirillum lipoferum (F S)	57.4	5.2	142	4.1
Pseudomonas sp.	58.5	4.5	132	10.9
Rhizobium ciceri	57.0	5.8	150	0.0
C.D. 5%	4.2	0.2	12.7	–

TABLE II - *Physiological properties of various strains of* Azotobacter chroococcum.

Strain/mutant	Ammonia excretion (μg ml^{-1})	IAA production (μM)	Siderophore
IS-16	14	180	ND
103	18	152	+
1(1)	14	91	+++
6(2)	21	398	ND
8003	14	26	ND
Mac 21	13	ND	+
Mac 27	10	ND	++
HT 54	28	ND	+

ND = Not detectable.

ever, the siderophores protect plants from other soil borne pathogens and deleterious rhizobacteria (Weller, 1988) and thus promote plant health.

Three strains of *A. chroococcum* namely, 103, HT 54 and 6(2) effectively reduced nematode infection by 40-60 per cent. All of the three strains are high ammonia excretors (K. Lakshminarayana, K. Kukreja and Neeru Narula, Unpublished data) suggesting that ammonia production may be involved in the biological control of *H. avenae* in wheat. Our results are in agreement with reports that both organic and inorganic nitrogen, especially ammonia, suppress nematode populations when applied to crops (Eno *et al.* 1955; Johnson and Shamiyeh, 1975; Kaplan and Noe, 1993). A previous investigation (Verma and Bansal, 1996) indicated that ammonium ions upto 50 ppm concentration had little effect on egg hatching of *M. javanica*, but Sudirman and Webster (1995) found that at higher concentrations (54 and 324 mg l^{-1}) of ammonium ions egg hatching of *M. incognita* was reduced and also that of root penetration by juveniles was inhibited. Ammonia seems to act by deterring juvenile penetration through diminished attractiveness of root tips as Castro *et al.* (1990) reported ammonium ions to be strongly repellent to *M. incognita* juveniles.

TABLE III - *Effect of various strains of* A. chroococcum *on biomass in nematode infected wheat.*

Strain/mutant	Average plant height (cm)	Average plant dry weight (g)	Nematode infection	
			Average no. of cysts	Reduction (%)
Control	58.0	5.3	0	–
Nematode only	52.0	4.2	148	–
Nematode + IS-16	53.6	6.0	133	10.2
Nematode + 103	64.8	6.3	60	59.5
Nematode + 1(1)	59.2	5.6	150	Nil
Nematode + 6(2)	54.4	5.1	83	44.0
Nematode + 8003	57.0	5.8	139	6.1
Nematode + Mac 21	52.0	5.5	117	21.0
Nematode + Mac 27	57.6	5.0	152	Nil
Nematode + HT 54	58.5	6.4	77	48.0
C.D. 5%	2.4	0.4	20.1	–

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