Supplement to Journal of Nematology 28(4S):682–686. 1996.

Population Growth of a Rhabditid Nematode on Plant Growth Promoting Bacteria from Potato Tubers and Rhizosphere Soil

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Abstract: Nine bacterial isolates from the rhizosphere and(or) tubers of potato (*Solanum tuberosum* cv. Kennebec) at a field site on Prince Edward Island were assessed as food sources for a bacteria-feeding nematode species tentatively identified as *Diplogaster lheritieri.* This species was the most common rhabditid nematode recovered from soil around potato roots at the same site. In laboratory feeding trials, an isolate of *Comamonas testosteroni* recovered from soil was an excellent food source for *D. lheritieri.* This bacterial isolate also increased the fresh weight and number of microtubers of tissue culture plantlets in the laboratory. Two endophytic bacterial isolates recovered from potato tubers, an *Agrobacterium* sp. and *Pseudomonas fluorescens,* were also good nutritional sources for the nematode. *Diplogaster lheritieri* spread bacteria over agar surfaces in petri plates.

Key words: Agrobacterium sp., bacteria, *Comamonas testosteroni,* culture regenerated plantlet, diet, *Diplogaster lheritieri,* nematode, plant growth promotion, potato, *Pseudomonas fluorescens,* Rhabditida, rhizosphere, *Solanum tuberosum,* tissue culture.

Samples of potato and rotational crop soils from the Maritime region of Canada usually contain, in addition to plant-parasitic nematodes, large numbers of several species of bacteria-feeding nematodes (13,14). Rhizosphere soil and the potato plant are often colonized by a variety of bacterial species that may or may not be harmful to the host plant (3), and some have been shown to promote growth (2,7, 15).

The relationships between bacteria and plant-parasitic nematodes, and the effects on plant hosts, have been discussed (17). However, there is little information on the effects of bacteria-feeding nematodes on the populations of endophytic or rhizobacterial species (1,18), and no data have been obtained previously in the Maritime region of Canada.

We assessed the effects of several bacterial isolates, recovered from a commercial potato field on Prince Edward Island, on the population development of a prevalent bacteria-feeding nematode species. The impact of these bacterial isolates on potato plantlets in the laboratory also was examined.

MATERIALS AND METHODS

Soil was collected at midseason (July) in a commercial potato (*Solanum tuberosum* cv. Kennebec) field on Prince Edward Island from the region adjacent to the seed piece and associated roots by shaking the adhering soil into a plastic bag. Bags with soil were transported in coolers to the laboratory and processed within 16 hours. Soil was plated onto soil extract agar (5), and individual bacteria colony forming units (CFU) were selected at random from the growth media and subcultured on potato dextrose agar (PDA) and tryptic soy agar (TSA).

At 110 days (September) after planting, tubers were harvested by hand and stored in the cold room at 4 to 5 °C for 6 weeks. Tubers from storage were sampled at random, washed in running water to remove soil, wiped with 95% ethanol, surface-sterilized in sodium hypochlorite (2% available chlorine) for 5 minutes, and rinsed three times in sterile distilled water. The surface-tension depressant polyoxyethylene sorbitan monooleate (Tween 80, Fisher Scientific, Fair Lawn, NJ) was used

Received for publication 27 October 1995.

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The authors thank P. Spence for technical assistance. This project was funded under the Canada-Prince Edward Island Agreement on Agri-Food Development.

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in the sterilant and rinsing solutions (1:1000 v/v, Tween 80:water). To verify that surface sterilization was successful, potato surface tissue was plated on five petri dishes of PDA or TSA.

Cylindrical plugs of potato tissue, 10-mm diam., were taken from 30 tubers through the stem-end (6) and rolled over the TSA surface in petri dishes. After incubation at 22 °C for 3 to 5 days, the bacterial CFU were selected at random and subcultured onto PDA and TSA. To ensure random and representative sampling of the bacterial flora, a line was drawn across the diameter of each petri dish and those CFU that were immediately adjacent to or bisected by the line were sampled. Differentiation of the bacterial genera was made using the protocols of Schaad (19); Bergey's manual of determinative bacteriology (10); and the Biolog 3N MicroStation, an automated bacterial identification system (RDG Laboratories, Hayward, CA).

Nine bacterial isolates were selected from within potato tubers and root zone soil for nematode feeding trials. Soil taken from the field site in late September shortly after harvest also was evaluated for the presence of bacteria-feeding nematodes using the modified Baermann funnel method (11). One gravid female of the most common bacteria-feeding nematode species was placed on a petri dish with PDA. After about 8 weeks, the plate contained >100,000 nematodes. Several hundred nematodes were removed from this population and placed in sterile tap water for 30 minutes, and then rinsed three times in sterile tap water. Each plate with a bacterial isolate was inoculated with five washed gravid female nematodes and incubated at room temperature (approximately 22 °C). Using a stereomicroscope at ×80 magnification, nematode counts were made 1, 2, 4, 7, 11, 14, and 17 days after inoculation. The entire plate was scanned, except when populations were large and the total number was estimated from subsections of the plate. Only one of the 36 petri plates used in the experiment showed visible signs of contamination and was discarded. Nematode counts were expressed as numbers of nematodes per plate, and the data were transformed by $\log_{10}(x + 1)$ before the analysis of variance. The design of the experiment was a randomized complete block with nine treatments and four replicates.

To determine the growth effects on potatoes, bacterial isolates were bioassayed in a series of inoculation trials using tissue culture regenerated plantlets grown from nodal cuttings in vitro in GA7 Magenta vessels on Musashige and Skoog Minimal Organic Media (Sigma Chemical Co., St. Louis, MO). Plantlets 6 to 7 cm in height were removed from their growth media, their roots washed in tap water, and placed in a hydroponic system containing commercial liquid fertilizer (20-20-20, NPK) in the greenhouse under a 16-hour photoperiod at 22 ± 2 °C. After 7 to 10 days of hydroponic growth, plantlets approximately 10 cm in height were selected, their roots washed in tap water, and inoculated with a suspension of one bacterial isolate in 25% strength Ringer's solution (8). Inoculated plantlets were immediately transplanted into individual Conetainers (4-cm × 20-cm pots tapering to 1 cm; Ray Leach Conetainers, Stuewe and Sons, Corvallis, OR) with pasteurized field soil and mixed with desalinated sand and commercial potting compost (2:1:1, soil:peat:sand). Controls included roots dipped in either water or 25% strength Ringer's solution.

Containers suspended in trays were arranged in a randomized complete block with four replicates per isolate. Container trays within the block were rotated every 1 to 2 days to remove any border effects. After 6 weeks, plants were removed and washed, and number of tubers as well as wet and dry weights of the shoots, roots, and tubers were recorded.

Results and Discussion

The bacteria-feeding nematode species recovered from rhizosphere of potato roots and propagated on the bacterial iso-

lates was tentatively identified as *Diplogaster lheritieri* (Maupas) (20). In the first few days after inoculation, sinusoidal trails of bacteria were present where nematodes had migrated along clear surfaces of the agar. This agreed with previous observations, which indicated that nematodes disperse bacteria by passing viable cells through the alimentary canal or by transporting cells that adhere to the cuticle (3, 4,9,12,17).

Three of the bacterial isolates, B52 (*Comamonas testosteroni*) from rhizosphere soil and B418 (*Pseudomonas* sp.) and B391 (*Agrobacterium* sp.) from tubers, were excellent energy sources for *D. lheritieri,* as indicated by the large numbers of nematodes in the petri plates at 17 days after inoculation (Table 1). When these isolates were present, nematode populations increased by at least 700-fold after 17 days. B52, B391, and B418 could also provide nourishment for *D. lheritieri* and other bacteria-feeding nematodes in the field (16). However, it is probable that the build-up of nematode numbers in the field would not be as great as in petri plates since the buffering action of other microorganisms in field soil would temper the effect.

Three bacterial isolates recovered from potato root zone soil—B62 (*Leuconostoc mesenteroides* subspecies *cremoris*), B66 (unidentified species), and B82 (*Pseudomonas fluorescens Type A*)—increased tuber dry weight ($P < 0.05$); B66 and B82 also increased the total wet weight ($P < 0.05$) of tissue-cultured plantlets in test tubes (Table 2). In addition, B52, recovered from potato root zone soil, increased the numbers of tubers and tuber wet weights of tissue-cultured plantlets in test tubes. When B52 was present, tuber fresh weight was 815 mg per plantlet and tuber number averaged 1.45 per container; when B52 was absent, tuber fresh weight and tuber number were lower ($P < 0.05$), at 104 mg per plantlet and 0.20 tuber per container, respectively. The remaining isolates did not affect plant growth.

Since *D. lheritieri* moved bacteria in agar plates and because the plant growth pro-

TABLE 1. Population development of *Diplogaster lheritieri* on nine bacterial isolates recovered from Kennebec potato (*Solanum tuberosum*) tubers or rhizosphere soil after inoculation with five gravid females to each plate.

Isolate	Species	Source	Mean number of *D. lheritieri* per plate Days after nematode inoculation						
			1	2	4	7	11	14	17
B52	*Comamonas testosteroni*	Soil	5	3	18	27	81	243	8,140
B418	*Pseudomonas* sp.	Tuber	5	5	9	27	63	217	3,709
B391	*Agrobacterium* sp.	Tuber	5	4	24	17	30	88	3,563
B436	*Pseudomonas fluorescens*	Tuber	5	2	3	3	5	5	5
B442	*Pseudomonas* sp.	Tuber	5	4	4	4	4	4	3
B66	Unidentified species	Soil	5	2	2	2	2	2	2
B82	*Pseudomonas fluorescens* Type A	Soil	5	2	2	<1	2	<1	<1
B345	*Agrobacterium* sp.	Tuber	5	1	2	1	<1	0	<1
B62	*Leuconostoc mesenteroides* ss *cremoris*	Soil	5	4	3	3	1	<1	0
SEM[a]			0	0.4	1.0	1.8	5.8	18.7	828.2

[a]Standard errors of the mean ($n = 4$, error df = 24).

TABLE 2. Effect of bacterial isolates from rhizosphere soil on growth of Kennebec potato (*Solanum tuberosum*) plantlets.

Isolate	Species	Source	Tuber dry weight mg/plant	Root wet weight mg/plant	Total wet weight mg/plant
B62	*Leuconostoc mesenteroides* ss *cremoris*	Soil	131	545	1,968
B66	Unidentified species	Soil	219	623	2,498
B82	*Pseudomonas fluorescens* Type A	Soil	249	512	2,808
Check			58	727	1,973
SEM[a]			28	35	185

[a]Standard errors of the mean (n = 4, error df = 9).

moter, B52, was the preferred diet in this study, it is possible that the nematodes could move viable cells around potato roots and help enhance plant growth in the field. If the growth-promoting capabilities of B52, which were evident in the laboratory, persisted in the field, then the spread of these isolates in the field might enhance potato production. However, there are many bacteria-nematode relationships that have not been investigated (17), and the same can be said for the association of bacterial species and *D. lheritieri* recovered from potatoes. With this in mind, experiments are planned to determine if the four bacterial isolates that stimulated plantlet growth in the laboratory interact with bacteria-feeding nematodes and enhance potato production in pots in the greenhouse and in experimental plots in the field.

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