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Bacterial-Feeding Nematode Growth and Preference for Biocontrol Isolates of the Bacterium *Burkholderia cepacia*

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Abstract: The potential of different bacterial-feeding Rhabditida to consume isolates of *Burkholderia cepacia* with known agricultural biocontrol ability was examined. *Caenorhabditis elegans, Diploscapter* sp., *Oscheius myriophila, Pelodera strongyloides, Pristionchus pacificus, Zeldia punctata, Panagrellus redivivus,* and *Distolabrellus veechi* were tested for growth on and preference for *Escherichia coli* OP50 or *B. cepacia* maize soil isolates J82, BcF, M36, Bc2, and PHQM100. Considerable growth and preference variations occurred between nematode taxa on individual bacterial isolates, and between different bacterial isolates on a given nematode. Populations of *Diploscapter* sp. and *P. redivivus* were most strongly suppressed. Only *Z. punctata* and *P. pacificus* grew well on all isolates, though *Z. punctata* preferentially accumulated on all isolates and *P. pacificus* had no preference. *Oscheius myriophila* preferentially accumulated on growth-supportive Bc2 and M36, and avoided less supportive J82 and PHQM100. Isolates with plant-parasitic nematicidal properties and poor fungicidal properties supported the best growth of three members of the Rhabditidae, *C. elegans, O. myriophila,* and *P. strongyloides. Distolabrellus veechi* avoided commercial nematicide M36 more strongly than fungicide J82.

Key words: accumulation, attraction *Caenorhabditis elegans, Diploscapter* sp., *Distolabrellus veechi,* ecology, *Escherichia coli* OP50, nutrition, *Oscheius myriophila, Panagrellus redivivus, Pelodera strongyloides,* phylogeny, *Pristionchus pacificus,* repellence, Rhabditida, toxicity, *Zeldia punctata.*

Bacterial-feeding nematodes are important for soil-nutrient cycling in agricultural systems (Freckman and Caswell, 1985). Plant productivity might be affected if applied biocontrol bacteria were toxic to these common non-target organisms. Alternatively, some soil bacterial-feeding nematodes may consume applied biocontrol bacteria. *Acrobeloides nanus* is known to feed on and reduce the field efficacy of the introduced bacterial-biocontrol agent, *Pseudomonas corrugata,* against *Gaumannomyces graminis,* the take-all fungus of wheat (Ryder and Bird, 1983).

Another agriculturally important bacterium applied as a biocontrol agent is *Burkholderia cepacia* (*ex* Burkholder) Yabuuchi, Kosako, Oyaizu, Yano, Hotta, Hashimoto, Ezaki and Arakawa, 1993 (= *Pseudomonas cepacia* (*ex* Burkholder) Palleroni and Holmes, 1981). Current taxonomic opinion suggests this is a polyphyletic species (Govan et al., 1996) where some isolates are plant or opportunistic-human pathogens (Hales et al., 1998). Isolates of this gram-negative bacterium are commonly found in soil and water. A number of biocontrol strains with excellent rhizosphere-colonizing ability have been isolated from maize rhizospheres (Hebbar et al., 1992a).

Burkholderia cepacia isolate M36 is registered with the U.S. Environmental Protection Agency as a nematicide (Stine Microbial Products, marketed by Market VI, L. L. C., Shawnee, KS) against plant-parasitic nematodes except for cyst nematodes (Noel, 1990) but has shown variable nematicidal activity (J. O. Becker, pers. comm.). *Burkholderia cepacia* isolates Bc2 (Meyer et al., 2000) and BcF (unpub.) reduced populations of root-knot nematodes. A soil isolate of *B. cepacia* that was not associated with maize supported good population growth of the bacterial-feeders *Acrobeloides* sp. (Cephalobidae) and *Pristionchus lheritieri* (Neodiplogastridae) (Anderson and Coleman, 1981; Anderson et al., 1981). However, nothing is known about the effects of maize-associated isolates with pesticidal properties on non-target bacterial-feeding nematode survival or bacterial

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consumption. Part of the variable plant-parasitic nematode control may be a result of bacterial-feeding nematodes using *B. cepacia* as an energy source. Nematicidal isolates, however, might kill or repel bacterial-feeders before the nematodes can consume substantial amounts of bacteria. Therefore, one objective of this study was to determine whether different *B. cepacia* isolates might differentially attract and support growth of different nematode species. The null hypothesis is that all isolates equally support growth and do not repel all bacterial-feeding nematodes.

None of the *B. cepacia* soil isolates tested so far have shown human-pathogenic potential (Hales et al., 1998), but the bacterium is known to be highly mutable and resistant to antibiotics. Under certain conditions, normally benign environmental *B. cepacia* strains might be transmissible to cystic-fibrosis patients. Because of this concern for *B. cepacia* strains in the agricultural environment (Holmes et al., 1998), nematodes that consume this bacterium may be useful agents for environmental remediation.

Better discrimination among agriculturally and medically important *B. cepacia* isolates and their modes of action might also be obtained from *Caenorhabditis elegans* mutants with pharmacologically interesting genes (Rand and Johnson, 1995). Therefore, a second purpose of this study was to quantify growth support of *C. elegans* on *B. cepacia* isolates as a basis for future genetic or toxicological tests. The population quantification also serves as a check on the reliability of the growth-rating scale for this nematode.

Materials and Methods

The bacterial-feeding nematodes used in the assays were selected to represent distinct groups within the Rhabditida based on molecular (Baldwin et al., 1997; Blaxter et al., 1998) and morphological criteria (Andrassy, 1984; Sudhaus, 1976). Nematodes used in the study were isolated and identified by the author in the current lab (LKC) or in the Sternberg lab (PS) at the California Institute of Technology, Pasadena, California, except as noted. They include *Oscheius myriophila* DF5020 (Rhabditidae: Rhabditinae) (from D. Fitch, New York University, New York, NY 10003); *Caenorhabditis elegans* N2 (Rhabditidae: Peloderinae) and *Panagrellus redivivus* PS1163 (Panagrolaimidae) (from the *Caenorhabditis* Genetics Center (CGC), University of Minnesota, 1445 Gortner Ave., St. Paul, MN 55108); *Diploscapter* sp. PS2123 (Diploscapteridae); *Pelodera strongyloides* PS1129 (Rhabditidae: Peloderinae); *Pristionchus pacificus* PS312 (Neodiplogastridae); *Distolabrellus veechi* LKC10 (Rhabditidae: Mesorhabditinae); and *Zeldia punctata* PS1192 (Cephalobidae). Voucher specimens are deposited in the U.S. Department of Agriculture Collection, Beltsville, Maryland.

All nematode stock cultures were grown at 20 °C on NGM agar spotted with *Escherichia coli* OP50 (CGC, St. Paul, MN) grown in L-Broth at 37 °C and transferred from a stock LB agar plate (Stiernagle, 1999). Besides its use for stock cultures, *E. coli* is used here experimentally as a bacterial control to compare population growth or relative preference with *B. cepacia* isolates.

All *B. cepacia* cultures used in this study were isolated from maize rhizospheres and have demonstrated biocontrol potential. These include antifungal isolates J82 (EPA registration no. 006419) (Mao et al., 1997), BcF (Mao et al., 1998), PHQM100 (Hebbar et al., 1998), and plant-parasitic nematode suppressive isolates M36 (nematicide, EPA registration no. 006464, Stine Microbials, Adel, IA) and Bc2 (Meyer et al., 2000). *Burkholderia cepacia* sources and alternate isolate designations with American Type Culture Collection (ATCC) numbers and Agricultural Research Service (USDA-ARS) numbers include (i) Stine Microbials, Adel, Iowa, for Wisconsin isolates J82 (alternate designations = ATCC 51993 or ARS BcB) and M36 (= ATCC 51995 or ARS B18379); (ii) D. P. Roberts and K. P. Hebbar, USDA, Beltsville, Maryland, for Maryland isolates ARS Bc2 and ARS BcF; and (iii) K. P. Hebbar for French isolate PHQM100.

Bacterial stock cultures were grown monthly on nutrient agar plates incubated for 24 hours at 30 °C. For experimental use,

most bacteria were grown for 24 hours in nutrient broths (Difco Laboratories, Detroit, MI) to achieve densities of 10^6 to 10^7 CFU/ml. Isolates Bc2 and M36 required an extra 12–24 hours to reach this density. *Burkholderia* strains were grown at 30 °C and *E. coli* at 37 °C.

Two types of experimental plates were made. Preference plates were prepared with opposing 30-µl spots of a *B. cepacia* isolate 6 cm away from a spot of *E. coli* OP50 on 2% water agar in 100-mm-diam. petri plates. Population growth plates were prepared with a central 30-µl spot of bacteria on 60-mm-diam petri plates of NGM agar. A stable bacterial lawn developed after 24 hours in a transfer hood (Stiernagle, 1999).

Quantitative C. elegans *population growth:* For each bacterial strain, five adults were placed on each of four growth plate lawns in an unlighted 20 °C incubator and two separate counts of all progeny stages made after 7 days ($n = 8$). The Kruskal-Wallis One Way ANOVA on median ranks with Tukey's pairwise multiple comparison test (SigmaStat 2.0, SPSS, Chicago, IL) were performed on the data.

Nematode population growth rating and individual appearance: Five adults were placed on bacterial lawns of 60-mm NGM agar plates and grown at 24 °C. This temperature supported good to optimum population growth of *Caenorhabditis, Pristionchus, Pelodera,* and *Acrobeloides* species on *B. cepacia* (Anderson and Coleman, 1981). At either 7 or 14 days the population growth plates were rated for nutritional status ($n = 2$). First, the qualitative appearance was rated by noting intestinal reserves of nematodes and condition of eggs. Then a population growth-rating scale was adapted to compare nematodes having different fecundity, growth rate, and mortality (Sohlenius, 1968; Andrew and Nicholas, 1976). In this scale, 4 = optimum population growth visually similar to that on *E. coli* plates, 3 = moderate growth of progeny population, 2 = poor growth of progeny, 1 = survival of any original five parents, and 0 = death. Death was determined by observing no movement and loss of intestinal reserves as evidenced by severely reduced optical density.

Preference plate tests: Nematodes were grown to adulthood on lawns of *E. coli* OP50 on NGM agar plates and rinsed once with sterile distilled water from plates recently depleted of bacteria to reduce interference with bacterial test spots. Within 15 minutes to an hour of rinsing, 50–100 adult nematodes were pipeted or individually moved with an eyelash to a drop of sterile water at the center of each test plate. Plates were discarded if an asymmetric distribution of nematodes in the central spot occurred due to uneven evaporation during the 15-minute inoculum drying time, or where at least one-third of the nematodes had not moved from the center spot at counting time. Counts were made of the number of nematodes in each opposing spot after 4 hours or 24 hours for the slower *P. strongyloides, Z. punctata,* and *Diploscapter sp.* The time intervals of the preference test allowed for the possibility of either taxis or random preferential accumulation. Any possible *E. coli* OP50 excreted from the nematodes would not grow on water agar because this bacterium requires uracil (Stiernagle, 1999). Nematodes accumulating in experimental bacterial spots were compared to *E. coli* OP50 control spots by calculating Attracting Activity Indices (AAI) (Ishikawa et al., 1986; Stamps and Linit, 1998). For this statistic, $AAI = 10 \times (N_t\text{-}N_c)/N_c$, where N_t = nematode numbers in treatment group, N_c = nematode numbers in control group, and the sign of the index reflects positive or negative accumulation for the treatment relative to the control. The Kruskal-Wallis One Way ANOVA on median ranks and Dunn's pairwise multiple comparison procedure were calculated (SigmaStat 2.0, SPSS, Chicago, IL).

Results

Nematode abnormalities were noted on all *B. cepacia* but not on any *E. coli* plates within 1 or 2 days of transfer. Eggs were often internally distorted and hyaline. Adults became sluggish and lost intestinal reserves.

Vulval eversion was common, and many adults died prematurely.

Quantitative C. elegans population growth: Seven days after inoculation of plates with *C. elegans* (approximately two generations), significant progeny population differences existed among the bacterial strains. For both experimental repetitions, progeny population statistics (median, range) indicated strain PHQM100 was least supportive of nematode growth, and increasing support occurred in the order BcF, J82, M36, Bc2, and *E. coli* OP50. Counts for OP50, Bc2, and M36 were greater ($P < 0.05$) than counts for J82, BcF, and PHQM100. The counts for J82 and BcF included many dead vermiform nematodes, and the PHQM100 counts included many unhatched eggs.

Population growth rating of bacterial-feeding nematodes: The growth of *C. elegans* on Bc2 and M36 was moderate to optimum (1,888 – 13,627 × and 2,443 – 19,080 × population range increase, respectively) and clearly better than the survival to poor growth on PHQM100 (14 – 406 × increase), J82 (574 – 921 × increase), and BcF (65 – 629 × increase) (Table 1). This discontinuous pattern was reflected in the growth-rating scale of Table 2 except there was a slightly wider range of growth from poor to optimum on Bc2 and M36. The rating scale for two other members of the Rhabditidae, *P. strongyloides* and *O. myriophila,* also had a similar discontinuous pattern. The pattern for *P. strongyloides* is like *C. elegans* except ratings are slightly lower. *Oscheius myriophila* had nearly the same pattern as *C. elegans* and *P. strongyloides* except for the better rating (2–4 vs. 1–2) on BcF (Table 2). All other nematodes had no discrete growth differences between bacterial isolates with this rating scale. *Pristionchus pacificus* and *Z. punctata* grew uniformly well on all isolates, while *P. redivivus* and *Diploscapter* sp. barely survived on them (Table 2). All isolates supported at least slight to moderate growth of *C. elegans, O. myriophila, P. strongyloides,* and *D. veechi,* and moderate to good growth of *P. pacificus* and *Z. punctata.* Of the eight tested nematodes, only *Z. punctata* and *P. pacificus* grew well on the PHQM100 isolate (Table 2). Within lawns of PHQM100, eggs of all nematodes had an unusual white halo around the egg cytoplasm.

TABLE 1. *Caenorhabditis elegans* population growth on *Escherichia coli* and *Burkholderia cepacia.*

Bacteria	Median	Range
E. coli OP50	44,358 A	15,848–108,500
B. cepacia Bc2	40,609 A	9,439–95,400
B. cepacia M36	38,725 A	12,216–68,136
B. cepacia J82	2,872 B	2,872–4,604
B. cepacia BcF	1,487 B	322–3,144
B. cepacia PHQM100	954 B	69–2,028

Five young adult nematodes were placed on each of four bacterial plates on separate dates at 24 °C. All living and dead progeny were counted after 7 days ($n = 8$). Medians, representing the final nematode population per plate, followed by the same letter are not different ($P \leq 0.05$) with Tukey's pairwise multiple comparison procedure. M36, Bc2, and BcF have demonstrated suppressive activity against plant-parasitic nematodes.

The nematode preference tests (Table 3) indicated PHQM100 was generally nonpreferred; however, *Z. punctata* had a strong preference (AII = 12.3). Similarly, J82 was not strongly preferred by any nematode except *Z. punctata* (AII = 6.9). *Distolabrellus veechi* avoided nematicide M36 more than fungicide J82 or fungal- and nematode-suppressive Bc2. Both *O. myriophila* and *Z. punctata* preferentially accumulated on geographically related Bc2 and BcF and on M36. However, *P. redivivus* preferentially accumulated on Bc2 but not BcF, and *P. strongyloides* and *Diploscapter* sp. preferentially accumulated on BcF but not Bc2.

Comparison of growth and preference: Rows in Tables 2 and 3 were compared for consistency of growth rating and preference. *Zeldia punctata* grew well and preferred all *B. cepacia* isolates, especially PHQM100. *Pristionchus pacificus* also grew well on PHQM100 and was the only nematode to preferentially accumulate rather than avoid it. The PHQM100 bacterial isolate supported the least growth or accumulation of all other nematode taxa. Though *P. pacificus* preferred *E. coli* to *B. cepacia,* it still grew well on all isolates of *B. cepacia. Oscheius myriophila* had the most consistent pattern of preferential accumulation to bacteria sup-

TABLE 2. Growth rating for *Burkholderia cepacia* isolates relative to *Escherichia coli* OP50.

Nematode	Bc2	M36	PHQM100	J82	BcF
1 week of growth					
Oscheius myriophila	3–4	3	0–2	0–2	2–4
Caenorhabditis elegans	2–4	2–4	1–2	1–2	1–2
Distolabrellus veechi	2–3	2–3	1–3	2–3	3
Pristionchus pacificus	4	2–4	3–4	3–4	3–4
2 weeks of growth					
Panagrellus redivivus	0–1	0–2	0–1	0–1	0–1
Diploscapter sp.	0–2	0–2	0–1	0–2	0–2
Pelodera strongyloides	2–3	3	0–2	2	1–2
Zeldia punctata	3–4	3–4	3–4	3–4	3–4

Five young adult nematodes and their progeny were grown on a lawn of bacteria over 1 or 2 weeks. Nematode population growth rating scale: 4 = optimum growth, 3 = moderate growth, 2 = poor growth, 1 = survival of five parents, and 0 = death. M36, Bc2, and BcF have demonstrated suppressive activity against plant-parasitic nematodes.

porting the best growth (Bc2, M36) and avoidance from less supportive bacteria (J82, PHQM100). Anomalous situations where nematodes avoided bacterial strains that supported moderate or good growth included *C. elegans* (AII = –5.1, growth rating = 2–4), *P. pacificus* (AII = –4.4, growth rating = 2–4) and *D. veechi* (AII = –8.6, growth rating = 2–3) on M36, *P. pacificus* (AII = –2.5, growth rating = 3–4) and *D. veechi* (AII = –2.8, growth rating = 2–3) on J82, and *D. veechi* (AII = –5, growth rating = 3) on BcF. The reverse situation where nematodes preferentially accumulated on isolates supporting poor growth occurred with *P. redivivus* toward Bc2 (AII = 21, growth rating = 0–1) and *Diploscapter* sp. (AII = 24, growth rating = 0–2) toward BcF.

DISCUSSION

The *B. cepacia* commercial fungicide J82 (Mao et al., 1998), fungicidal PHQM100 (Hebbar et al., 1998), and both fungicidal (Mao et al., 1998) and nematicidal (unpubl. data) BcF suppressed bacterial-feeding nematodes more strongly here than the commercial nematicide M36 or the *M. incognita*-suppressive Bc2 (Meyer et al., 2000). The commercial nematicide M36 is not so suppressive or repellent that bacterial-feeding nematodes could not consume it as might have been predicted based on nematicidal properties. Instead, most bacterial-feeders readily consumed it (Tables 1,2). However, all but *O. myriophila* and *Z. punc-*

TABLE 3. Pairwise nematode preference for *Burkholderia cepacia* isolates relative to *Escherichia coli* OP50.

Nematode	*B. cepacia* Bc2	*B. cepacia* M36	*B. cepacia* PHQM100	*B. cepacia* J82	*B. cepacia* BcF	*E. coli* OP50
4 hours						
Oscheius myriophila	30.0 A	9.2 AB	–2.0 B	1.2 B	2.2 AB	0.0 AB
Caenorhabditis elegans	3.5 A	–5.1 AB	–5.2 AB	–6.1 B	–3.4 AB	0.0 AB
Distolabrellus veechi	–2.0 AB	–8.6 C	–6.2 BC	–2.8 AB	–5.0 ABC	–0.1 A
Pristionchus pacificus	0.0 A	–4.4 A	–0.8 A	–2.5 A	–1.5 A	0.1 A
Panagrellus redivivus	21.0 A	–6.7 B	–4.5 B	1.7 AB	–5.5 B	0.0 B
24 hours						
Diploscapter sp.	–0.2 A	0.0 A	–8.6 B	–0.2 AB	24.2 A	1.5 A
Pelodera strongyloides	–3.2 B	–4.8 AB	–4.7 B	–5.2 B	37.0 A	–1.8 AB
Zeldia punctata	6.7 AB	3.1 AB	12.3 A	6.9 AB	4.3 AB	0.0 B

Attracting Activity Index $(N_t\text{-}N_c)/N_c) \times 10)$, where N_t = number in treatment group and N_c = number in control; medians of positive attraction or negative repulsion in a row with common uppercase letters are not different ($P \leq 0.05$) with Dunn's pairwise multiple comparison procedure. Fifty to one hundred nematodes per plate. M36, Bc2, and BcF have demonstrated suppressive activity against plant-parasitic nematodes.

tata preferred to accumulate on *E. coli* rather than M36. The M36 nematicide label claims repellence as a mode of action against plant-parasitic nematodes. Although it is not clear that the same repellent factors are involved against bacterial-feeders, *D. veechi* showed less ($P < 0.05$) accumulation on M36 than fungicidal J82. Growth-suppressive isolates PHQM100 and J82 also were not preferred by any nematode except *Z. punctata*.

The enhanced growth of *Z. punctata* and *P. pacificus* on the *B. cepacia* isolates in this study was observed previously for *Acrobeloides* sp. and *Pristionchus Iheritieri* (Anderson and Coleman, 1981; Anderson et al., 1981). These nematodes belong to phylogenetic outgroups of the Rhabditidae based on both molecular and morphological criteria (Baldwin et al., 1997; Maggenti, 1981). Both are relatively unaffected by these biocontrol isolates despite their closer relationship to plant-parasitic Tylenchida (Baldwin et al., 1997). Another interesting issue is the similarity in poor growth support for both *C. elegans* and *Diploscapter* sp. These nematodes were recently shown to have a close molecular relationship (Blaxter et al., 1998) despite greater morphological similarity of *Diploscapter* to *Z. punctata* and other Cephaloboidea. A phylogenetic transition from poor support in Rhabditidae (*O. myriophila, C. elegans, P. strongyloides*) and Diploscapteridae (*Diploscapter* sp.) to good growth in molecular and morphological outgroup members *D. veechi, P. pacificus,* and *Z. punctata* was best demonstrated with isolate J82, and less clearly with BcF. On BcF, the growth rating of *D. veechi* is similar to *O. myriophila*. The exception to this phylogenetic trend was *P. redivivus,* the only member of a Rhabditidae outgroup consistently suppressed by all *B. cepacia* isolates.

Different isolates of *B. cepacia* may have the potential to affect nematode communities. The procedure and range of nematode responses in this study provide a preliminary frame of reference for what might occur with other nematode-bacteria combinations in the laboratory and field. In the field, it may be useful to test potential biocontrol bacteria with the particular bacterial-feeding nematodes present in the targeted area. Differences in nematode response to a native, possibly attractive *B. cepacia* strain might be sufficiently different from an introduced, possibly repellent strain. Combinations of attractive and repellent isolates on combinations of economic, cover, and trap crops might also prove useful. A repellent strain of bacteria on a legume might reduce the damage a nematode such as *Acrobeloides* could do to the internal *Rhizobium* bacteroids in pea roots (Westcott and Barker, 1976). In the laboratory, similar studies with other biocontrol bacteria may be used to select an experimentally appropriate bacterial-feeding nematode to screen bacterial isolates for growth, motility, or preference. For these bacterial isolates, *O. myriophila* has an intermediate capacity for differentiating relative growth suppression, while *P. redivivus* is very sensitive and *P. pacificus* is very resistant.

Although nutritional insufficiency could account for suppressed nematode growth by these bacteria, enzymes and toxins may also be involved in these effects. The eggs of all nematode species were often deformed in the presence of *B. cepacia*. This observation may be related to the suppression of egg hatch of the plant parasite *M. incognita* treated with Bc2 cell-free filtrate (Meyer et al., 2000). Nematode eggshells contain chitin (Bird and Bird, 1991) and, though *B. cepacia* may increase in chitin-amended soil (Hallmann et al., 1999), chitinase was not detected in the isolates used here (Meyer et al., 2000). However, nematode eggs contain significant amounts of lipid under the chitinous layer (Bird and Bird, 1991), and Hebbar et al. (1992b) found that nearly 70% of *B. cepacia* strains associated with maize had lipase activity. Several known *B. cepacia* toxins (Homma et al., 1989) may also have contributed to egg-hatch-suppression or other defects in these nematode species. These isolates, or any of their toxins, could be screened with genetic mutants of *C. elegans* to identify genetic pathways or physiological systems that may be adversely affected (Rand and Johnson, 1995). Highly suppressive bac-

terial isolates or their toxins that may suppress growth of other rhabditid taxa (J82, BcF, PHQM100) may be used to screen for anomalously sensitive species in members of the Cephalobidae and Neodiplogastridae. These species might then be further studied to better understand toxin-sensitive nematode genes.

Bacterial-feeding nematodes are neither uniformly supported by biocontrol isolates of *B. cepacia* nor uniformly killed or repelled. Variation occurs between nematode taxa on a given bacterial isolate, and between different bacterial isolates on a given nematode. Systematically selected bacterial-feeding nematodes might be useful bioindicators in future bacterial biocontrol studies.

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