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Feeding of the Nematode *Acrobeloides nanus* on Bacteria

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Abstract: Information on the effect of bacteria-feeding nematodes on bacterial populations in the soil is sparse. We have isolated, cultured, and microscopically examined bacteria and nematodes coexisting within an agricultural soil and have studied their feeding relationship. The bacterium *Pseudomonas corrugata* isolate 2140R is a biocontrol agent against the pathogenic fungus *Gaeumannomyces graminis* var. *tritici*. The nematode *Acrobeloides nanus* is a cosmopolitan, bacteria-feeding organism widespread in agricultural and arid soils throughout Australia. Using light and electron microscopy, we observed the ingestion and breakdown of *P. corrugata* in the pharynx of *A. nanus* and bacterial passage through the nematode intestine as well as the accumulation of fluorescent compounds from ingested and broken *P. fluorescens* in the lumen of the nematode's intestine. We also observed *A. nanus* feeding, growing, and reproducing on the Gram-positive bacterium *Clavibacter toxicus*, the causative agent of the disease annual ryegrass toxicity, and detected crushed bacteria in the nematode's intestine.

Key words: Acrobeloides nanus, bacteria, biological control, *Clavibacter toxicus*, feeding, nematode, *Pseudomonas corrugata*, *P. fluorescens*, ultrastructure.

The bacterium *Pseudomonas corrugata* functions as a biocontrol agent against the pathogenic fungus *Gaeumannomyces graminis*, which causes "take all" disease of wheat (13). This fungus, which grows in the roots of wheat, can reduce the yield of wheat in South Australia by as much as 75% (12). For this reason, there has been considerable interest shown in the selection and testing of bacterial biocontrol agents.

One aspect of this research that has not received attention is the influence of bacteria-feeding nematodes on these bacteria. Clearly, such a nematode would occur naturally in the same environment as the bacterium, survive under the same environmental conditions as the bacterium and, of course, would feed on the bacterium. In this paper, we describe the isolation, identification, culturing, and feeding of *Acrobeloides nanus* and identify and trace the breakdown of bacteria in the gut of the nematode.

MATERIALS AND METHODS

The nematode: The soil sample that was the source of nematodes had originally been collected from Wagga Wagga in New South Wales and stored dry for 5 years. This particular sample was chosen because it was the source of the parent strain of *P. corrugata* used in these experiments. Various species of living nematodes were isolated from the dry soil sample by incubation in a misting apparatus for several days. These nematodes were not only in the anhydrobiotic state but also shared the same environment as the bacterial parent strain. The most numerous and easily recognized of these nematodes was a small, round-tailed nematode readily cultured on *P. corrugata* growing on various media: mainly Nutrient Agar (NA, Difco) and Malt Extract Agar (Oxoid). This nematode was identified as *Acrobeloides nanus* (de Man) Anderson, a member of the Cephalobidae.

Bacteria: The nonfluorescent *Pseudomonas corrugata* isolate 2140 was originally isolated from the rhizospheres of wheat (*Triticum aestivum*) seedlings grown in soil from Wagga Wagga (13). Strain 2140R was isolated as a spontaneous mutant resistant to rifampicin (100 μg/ml) growing on nutrient agar by J. Brackin, Monsanto Co., St. Louis, USA. The fluorescent bacterium *Pseudomonas fluorescens* strain 2-79 (ATCC B15132) was originally obtained from Dr. D. M. Weller, USDA ARS, Pullman, Washington. These pseudomonads were routinely cultured on Nutrient Agar (NA,

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Difco). *Pseudomonas fluorescens* exudes fluorescent compounds when grown in a medium containing low available Fe^{+++}, such as medium B of King et al. (7). *Clavibacter toxicus* was originally isolated from bacterial seed galls of ryegrass (*Lolium rigidum*). The strain CS14 (4) was grown in a medium consisting of the following (in grams per liter): sucrose, 10; casein hydrolysate, 2; yeast extract, 2; K_2HPO_4, 2; $MgSO_4 \cdot 7H_2O$, 0.3; agar, 15. Cross-contamination was avoided by repeated washing of the nematodes in sterile distilled water (SDW), by using rifampicin-resistant pseudomonad strains, and by utilizing the resistance of *C. toxicus* to nalidixic acid (30 μg/ml).

Light microscopy: Living nematodes were examined at 25× or 50× in agar in sealed petri dishes or in SDW under a coverslip sealed at its edges with nail varnish. Observations were made with a Vanox Olympus (Tokyo, Japan) AHBT research microscope using transmitted light for bright field and interference contrast optics and incident light for fluorescent microscopy using the AHZ-RFL attachment. The filter sets consisted of a DM400 dichroic mirror, an UG1 excitation filter, and an L420 barrier filter for UV fluorescence and a DM500 dichroic mirror, a BP490 excitation filter, and an O515 barrier filter for blue light fluorescence. Photographs were taken with Ilford Delta 400 black-and-white film and Fujichrome 100 daylight color film using a LBD filter.

Electron microscopy: Specimens of *Acrobeloides nanus* cultured on *P. corrugata* were washed with SDW from culture dishes, manually picked from the water, and placed in a small amount of cold SDW in a dish surrounded by ice. Phosphate-buffered (pH 7.3, 0.1 M) 4% paraformaldehyde at 0 C was added to create a 3% paraformaldehyde solution, and the resultant nematode mixture was placed in a sealed dish at 8 C in a refrigerator for 2 days before being removed to room temperature (22 C).

The nematodes were placed in SDW and cut in half with a fine scalpel in order to facilitate the entry of stains, dehydrants, or embedding media. They were placed in 2% aqueous osmium tetroxide for 2 hours at 22 C, followed by three washes in SDW at evenly spaced intervals over 2 hours. The specimens were then passed through a graded ethanol series with two final washes in absolute ethanol, ethanol:propylene oxide (1/1), propylene oxide, propylene oxide:TK3 resin (TAAB Laboratories Equipment, Aldermaston, England) (1/1), and pure TK3 in vacuo overnight. Specimens were then polymerized in fresh TK3 at 60 C for 24 hours.

Sections were cut with a diamond knife in a Reichert-Jung Ultracut E ultramicrotome at thicknesses of 50 nm and 55 nm to yield silver and gold interference colors, respectively. These sections were mounted on uncoated grids, stained in freshly prepared aqueous 2% uranyl acetate for 1 hour at 22 C, washed six times in SDW, stained again in freshly made 0.5% lead citrate in 0.1 M sodium hydroxide for 15 minutes at 22 C, and washed six times with SDW.

The sections were examined and photographed in a Philips (Eindhoven, The Netherlands) EM 400 transmission electron microscope at 80 kV. The examined bacteria were treated similarly, except they were either cut from agar plates in blocks or scraped from the surface of agar plates and centrifuged in eppendorf tubes at 15,000*g* for 2 minutes between treatments.

Results

The *Acrobeloides nanus* isolated from the Wagga Wagga soil grew and reproduced on *Pseudomonas corrugata* (ca. 0.4 × 2.4 μm), *P. fluorescens*, and *C. toxicus* (ca. 0.7 × 1.5 μm). Great care was taken by washing and plating to obtain sterile *A. nanus* and prevent cross-contamination of bacteria. Tangential sections of *P. corrugata* before (Fig. 1) and after ingestion by *A. nanus* revealed bacteria in the muscular pharyngeal bulb being broken, so that only the extruded contents and the bacterial membranes remained (Fig. 2). After being

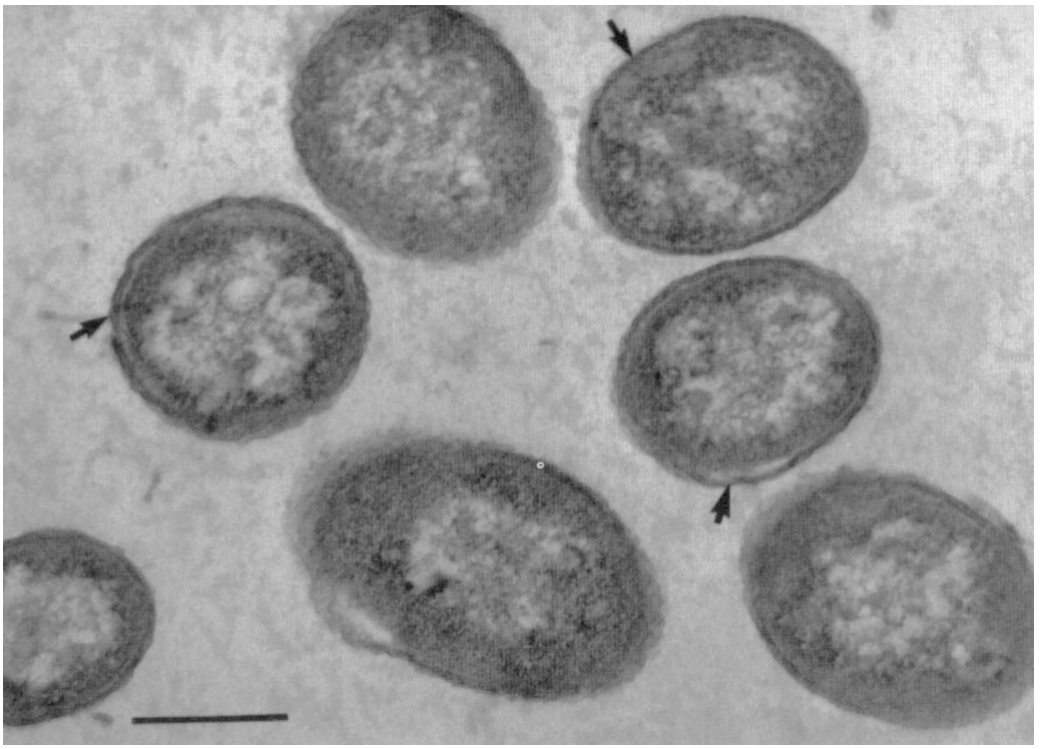

FIG. 1. Transmission electron micrograph of sections of *Pseudomonas corrugata*. Arrows point to bacterial membranes in transverse sections. Bar = 0.25 μm.

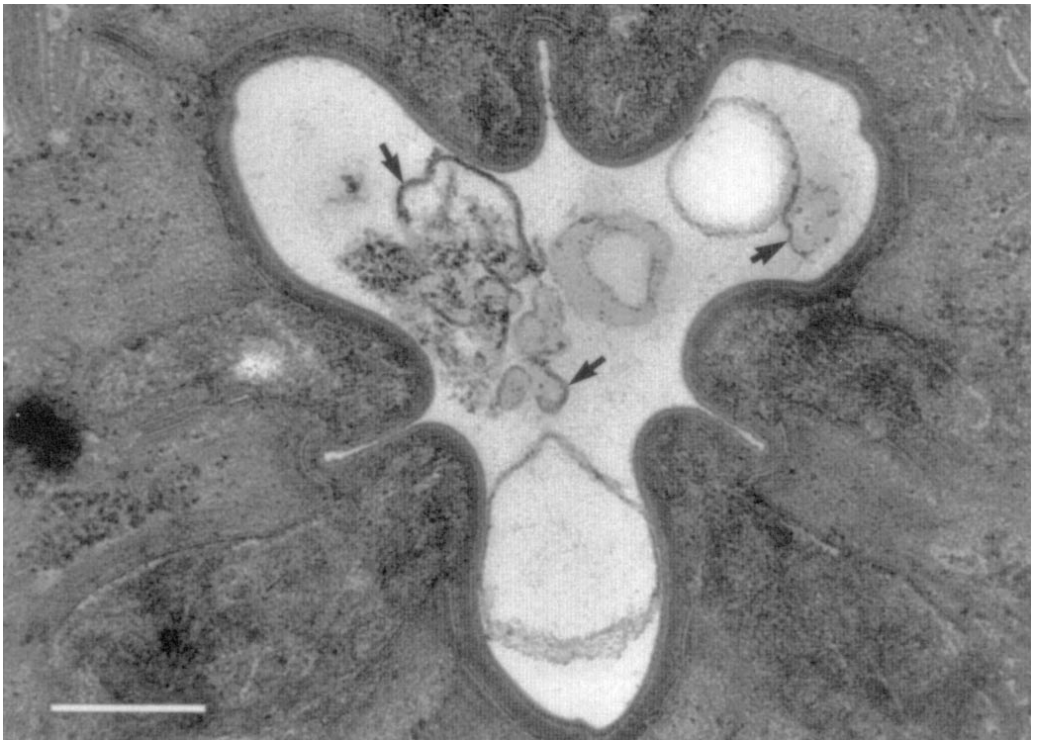

FIG. 2. Transmission electron micrograph of a transverse section through the triradiate pharynx of *Acrobeloides nanus* feeding on *Pseudomonas corrugata* and containing crushed and broken bacteria in the lumen. Bar = 0.25 μm.

crushed, the bacterial remains passed into the intestine (Fig. 3). This digestive process in *A. nanus* was obvious during light microscopy when *P. fluorescens* grown on King medium B was the food source. Under these conditions, the bacterium, although not fluorescent itself under UV or blue light, secreted a pyoverdin siderophore that did fluoresce. When the bacterium was crushed, its contents were invisible under bright field (Fig. 4A) but fluoresced under blue or UV light and became visible in the lumen and intestinal cells in both larvae (Fig. 4B) and adults. Although some granules in the intestinal cells of *A. nanus* autofluoresced when grown in all other media used, which did not cause the bacteria to produce fluorescent substances, this fluorescence was insignificant compared to that of the pyoverdin-secreting bacteria fed to the nematode (Fig. 4B).

As well as feeding on the Gram-negative pseudomonads, *A. nanus* also grew and reproduced on the Gram-positive bacterium *Clavibacter toxicus* strain CS14. However, the nematode did not appear to break down this bacterium as easily as it did the pseudomonads. When sections cut through *A. nanus* fed on *C. toxicus* were examined under the TEM, *C. toxicus* was surrounded by a capsule about 0.1 μm in thickness (Fig. 5). This capsule, although crushed in some bacteria (Fig. 5), remained intact in others; thus, the capsule may confer some mechanical protection to the bacterium.

Discussion

Acrobeloides nanus occurs in Canada (1) and Sweden (6), and it is also widely distributed in both agricultural and arid soils in Australia (8). The fine structure of *C. toxicus* was first described 16 years ago (4),

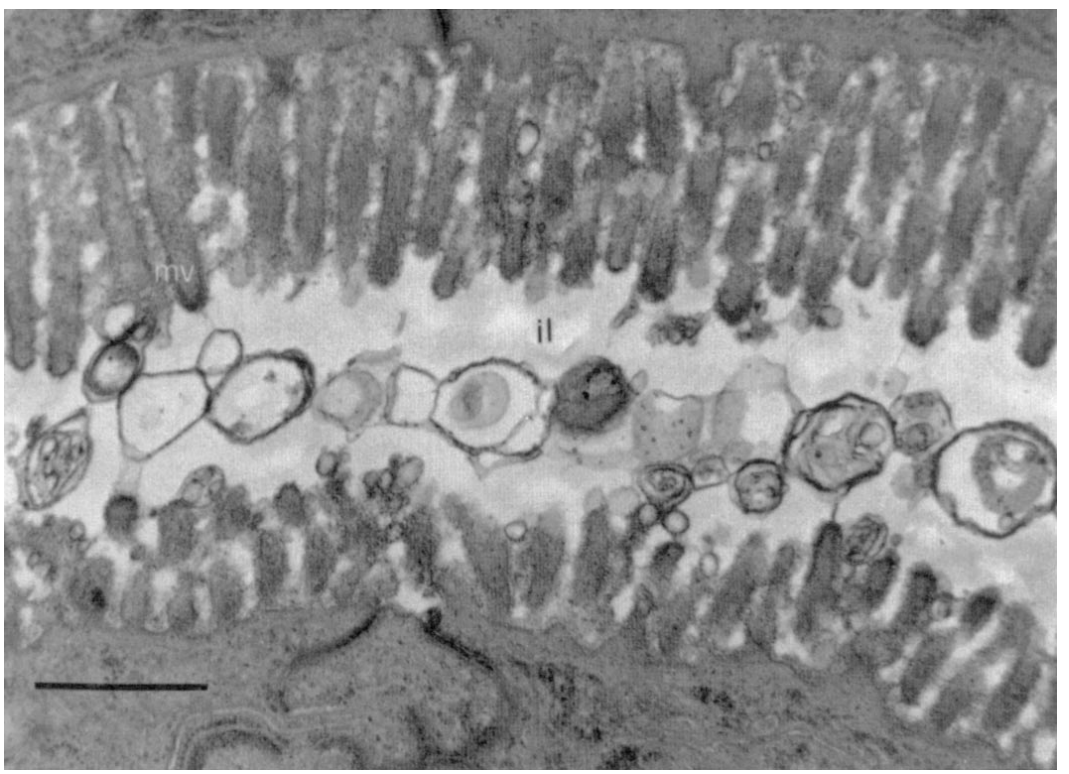

Fig. 3. Transmission electron micrograph of a tangential section through the intestinal lumen of *Acrobeloides nanus* feeding on *Pseudomonas corrugata*, showing numerous tangentially cut microvilli (mv), and a string of crushed bacteria in the intestinal lumen (il). Bar = 0.5 μm.

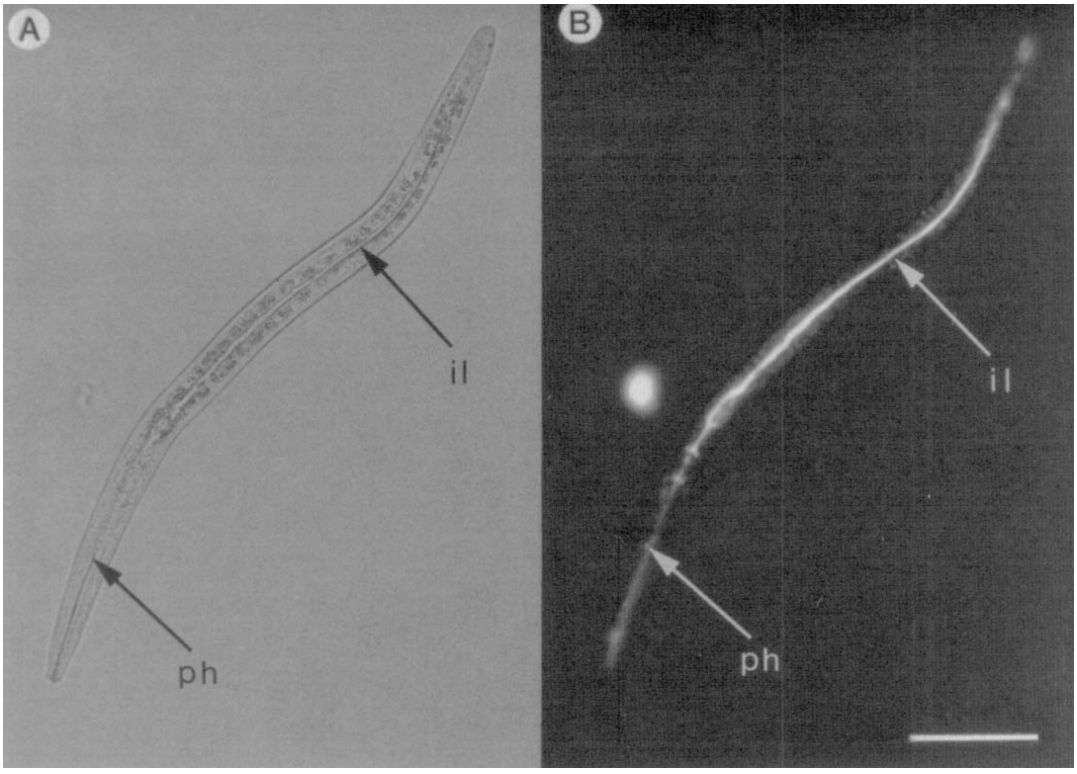

FIG. 4. Living fourth-stage *Acrobeloides nanus* feeding on *Pseudomonas fluorescens* and observed under (A) normal bright field transmitted light and (B) incident ultraviolet light. Note that the contents of the pharyngeal lumen (ph) weakly fluoresce whereas the contents of the intestinal lumen (il) strongly fluoresce. Bar = 50 μm.

and that of *P. corrugata* is shown in this paper. Both of these bacteria and *A. nanus* can survive in the soil during the hot Australian summer. Nicholas and Stewart (8) showed that an Australian population of *A. nanus* can survive as an anhydrobiote for years in this state and has the capacity to be dispersed over great distances by dust storms and high winds. It is not surprising, therefore, that this nematode is distributed so widely over the earth.

Although associations between plant-parasitic nematodes and bacteria have received some attention (2,3,5,9,10,14) surprisingly little information is available on associations between specific bacteria-feeding nematodes and the specific bacteria upon which they feed in the soil.

We believe that the anhydrobiotic capacity of *A. nanus* under field conditions is an essential prerequisite for its use as a stable tool for evaluating the long-term effectiveness of a range of bacterial biological control agents of fungi in the soil. We speculate that an increase in yield may arise through the promotion of plant growth by an increase in the availability of nutrients in the rhizosphere as a result of the breakdown of a range of rhizosphere-inhabiting bacteria by *A. nanus* and the release of the bacterial contents into the soil. This release is associated with the regular and rapid rates of defecation of many bacteria-feeding nematodes (3).

Fluorescent pseudomonads play a role in the suppression of fungal diseases of plants (16). The effect that nematodes that feed on these pseudomonads have on this biological control has not been tested, but *A. nanus* can decrease the numbers of *P. corrugata* in pasteurized soil to which both have been added (Ryder & Bird, unpubl.). This might partly explain the observation of Weller (15) that the number of intro-

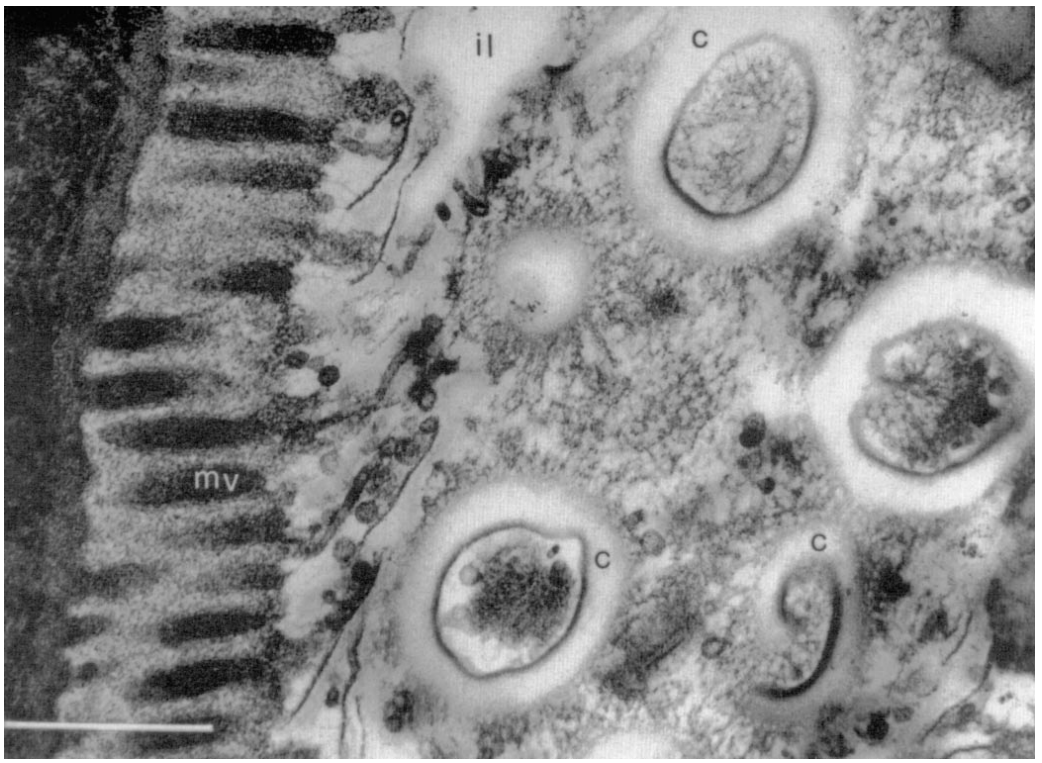

FIG. 5. A section through the intestinal lumen of *Acrobeloides nanus* fed on *Clavibacter toxicus*, showing microvilli (mv) and crushed and whole bacteria with their thick capsules (c) in the nematode's intestinal lumen (il). Bar = 0.5 μm.

duced fluorescent pseudomonads in the field decrease over time.

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