Saprozoic Nematodes as Carriers and Disseminators of Plant Pathogenic Bacteria[1]

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Abstract: The plant pathogenic bacteria *Agrobacterium tumefaciens* (Smith and Townsend) Conn. (strain 5–14 Deep), *Erwinia amylovora* (Burill) Winslow *et al.*, *E. carotovora* (Jones) Holland and *Pseudomonas phaseolicola* (Burk.) Dows. (ICPB-PM3) and the red-pigmented non-pathogen *Serratia marcescens* Bizio were hosts for the saprozoic nematode *Pristionchus lheritieri* (Maupas, 1919) Paramonov. Viable bacteria survived passage through the nematode and produced typical colonies on nutrient agar plates. Female nematodes ingested more bacterial cells and retained them longer than did males. It was hypothesized saprozoic nematodes may disseminate pathogenic bacteria to new infection courts.

Most investigators conclude that saprozoic nematodes feed on bacteria or by-products of bacterial metabolism. Occurrence of this group of nematodes in association with decomposing plant tissue or in soil samples is commonly recognized. Interrelationships of saprozoic nematodes and plant pathogenic bacteria occasionally have been reported. For example, Steiner (3) suggested in 1933 that a saprozoic nematode, *Pelodera* (*Rhabditis*) *lambdiensis* (Maupas) Dougherty may carry a mushroom pathogen, *Pseudomonas tolaasii* Paine, in its pharynx or intestine. Jensen (2) recently reported *Pristionchus* (*Diplogaster*) *lheritieri* (Maupas) Paramonov, *Panagrellus redivivus* (Linn.) Goodey, *Panagrolaimus subelongatus* (Cobb) Thorne, and *Rhabditis* spp. ingested and voided viable spores of various plant pathogenic fungi and a plant pathogenic bacterium, *Pseudomonas syringae* Van Hall.

Ingestion and voiding (defecation) of viable phytobacteria by saprozoic nematodes may be of some significance in dissemination and survival of bacterial pathogens. This investigation is concerned with associations of a saprozoic nematode and 4 species of plant pathogenic bacteria.

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MATERIALS AND METHODS

Pristionchus lheritieri, originally isolated from a decaying carrot approximately three years ago and maintained in the laboratory on cultures of *Pseudomonas* sp., was selected as the test vector. Pure cultures of *Agrobacterium tumefaciens* (Smith and Townsend) Conn Strain 5–14 Deep (obtained from I. W. Deep, Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon), *Erwinia amylovora* (Burrill) Winslow *et al.* (obtained from Carolina Biological Supply Company, Powell Laboratories Division, Gladstone, Oregon), *Erwinia carotovora* (Jones) Holland (obtained from Carolina Biological Supply Company, Powell Laboratories Division, Gladstone, Oregon), *Pseudomonas phaseolicola* (Burk.) Dows. ICPB-PM3 (obtained from M. P. Starr, Curator, International Collection of Phytopathogenic Bacteria, University of California, Davis, California) were used as test organisms to demonstrate ingestion and survival.

The following is a brief account of a method developed to exchange the natural bacterial flora of the nematode's alimentary tract as suggested by Chantanao (1). Gravid females were surface-sterilized 20 min in 20

TABLE 1. Plate counts of *Agrobacterium tumefaciens* ingested and defecated during 24 hr by female *Pristionchus lheritieri*, after various periods of starvation.

Period of Starvation (hr)	Number of Bacteria Per Nematode[a]					Defecation of Bacteria
	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Mean	
0	49,000	43,625	32,250	57,000	46,219	+
3	25,375	34,750	27,875	33,625	30,406	+
6	21,000	24,250	9,375	8,750	15,844	+
9	8,712	6,987	7,262	7,237	7,550	+
12	6,812	6,100	6,700	5,600	6,303	+
15	5,350	4,675	5,225	4,862	5,028	+
18	2,550	3,850	2,975	3,637	3,252	+
21	1,437	1,837	1,987	1,925	1,797	+
24	364	402	291	252	327	+
27	101	107	92	122	106	+
30	44	19	54	36	38	−
33	0	0	0	0	0	−

[a] Average of four nematodes each.

ppm chlorine then transferred to plates of nutrient agar (3 g of beef extract, 5 g of peptone, 15 g of agar in one liter of distilled water) containing 1000 ppm of tetracycline hydrochloride. Eggs deposited on these plates were transferred to nutrient agar plates containing the red-pigmented bacterium, *Serratia marcescens* Bizio (obtained from H. Hayes, Department of Microbiology, Oregon State University, Corvallis, Oregon). This bacterium is utilized to indicate contamination when other than red colonies occur. Gravid females from the *S. marcescens* plates were surface-sterilized and transferred to nutrient agar plates containing tetracycline hydrochloride as previously described. Eggs from these plates were placed upon nutrient agar plates. If no carry-over contamination (indicated by red colonies) was observed, emerging larvae were transferred to a plate containing different bacteria to complete the flora exchange.

A different procedure was used to determine the number of *Agrobacterium tumefaciens* ingested and the survival time of this bacterium in the alimentary tract. Accession time in *A. tumefaciens* cultures varied depending upon the type of experiment. Nematodes used to determine the survival time of ingested bacteria were limited to a standard feeding period of 24 hr. After feeding, the nematodes were removed, surface-sterilized 20 min in 20 ppm chlorine and transferred to nutrient agar plates containing tetracycline hydrochloride. Many bacteria, including test bacteria, were unable to grow in this medium thus eliminating the source of additional food for the nematodes. At 3-hr intervals several males and females were removed and placed in 20 ppm chlorine for 20 min. Following this treatment, four replicate groups (each containing four nematodes) of males and of females were transferred to nutrient agar plates (one plate for each group). Similar groups were aseptically crushed in 1 ml distilled water, the volume increased to 10 ml and 1 ml carried through a standard dilution series (10^{-2} to 10^{-5}) transferred to duplicate nutrient agar plates. Colony counts were made after incubation 48 hr at 25 C.

RESULTS AND DISCUSSION

Pristionchus lheritieri from laboratory cultures upon *Pseudomonas* sp. thrived on the five different bacteria including the red-

TABLE 2. Plate counts of *Agrobacterium tumefaciens* ingested and defecated during 24 hr by male *Pristionchus lheritieri*, after various periods of starvation.

Period of Starvation (hr)	Number of Bacteria Per Nematode[a]					Defecation of Bacteria
	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Mean	
0	10,375	7,615	12,375	9,375	9,937	+
3	376	384	522	680	491	+
6	187	171	235	252	211	+
9	79	156	112	141	122	+
12	12	16	32	39	25	−
15	0	0	0	0	0	−

[a] Average four nematodes each.

pigmented *S. marcescens* and the plant pathogens *A. tumefaciens, E. amylovora, E. carotovora* and *P. phaseolicola*. At least some bacteria of all species survived passage through the nematode's digestive tract and produced typical growth upon nutrient agar plates. After feeding 24 hr, female nematodes defecated viable *A. tumefaciens* and *P. phaseolicola* for an additional 27 hr and *E. amylovora* and *E. carotovora* were defecated for 21 hr. Males did not defecate viable bacteria after 9 hr.

Interrelationship studies of *P. lheritieri* and the crown gall bacterium indicated ingestion of an average of about 46,000 bacterial cells in 24 hr by female nematodes (Table 1) and 10,000 bacterial cells by males (Table 2). The smaller number ingested by males probably reflects their smaller size and food requirements as well as less aggressive feeding habits. In either sex, the maximum accumulation of bacterial cells occurred during the initial 24-hr feeding period. In carrier nematodes denied access to food by placing them on antibiotic-supplemented media, the number of recoverable bacterial cells decreased rapidly to zero. Thus survival or persistence in the carrier nematode was limited to 30 hr in the female (Table 1) and 12 hr in the male (Table 2). A prolonged cessation of the food supply interrupted defecation and probably many other body functions which may affect the persistence of bacteria in the carrier nematode.

Bacteria or by-products of bacterial metabolism support enormous populations of saprozoic nematodes in laboratory cultures. Although numerous typical colonies grew from defecations, we did not determine the fate of all ingested cells including those probably used for sustenance. The superabundance of bacteria available for food in these experiments may have exceeded the digestive capacity of the nematode thus allowing more cells to pass through unharmed than if fewer bacteria were ingested.

Nematodes also can disseminate bacterial pathogens which adhere to their external body surface as well as by ingestion and defecation. Thus the important rôle of saprozoic nematodes in this interrelationship appears to be one of dissemination.

LITERATURE CITED

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