Transmission of an *Agrobacterium tumefaciens* Phage by *Pristionchus lheriteiri*[1]

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Abstract: Pristionchus lheriteiri (Maupas) Paramonov, a saprozoic nematode, served as a carrier of an unnamed phage of *Agrobacterium tumefaciens* (Smith and Townsend) Conn. Viable phage particles passed through the nematode, caused lysis and formed typical plaques on agar plates seeded to *A. tumefaciens*. Phage retention by carrier nematodes was extended several hr by restricting food intake. Female nematodes accumulated phage in greater quantities and more rapidly than male nematodes.

Interrelationships between nematodes and viruses were unknown until 1941 when Shope (7) discovered swine lungworm to be a vector of swine influenza virus. A similar relationship between a plant-parasitic nematode (*Xiphinema index* Thorne and Allen) and a plant virus (grape fanleaf virus) was reported in 1958 (5).

In 1960 Chang *et al.* (3) showed two nematodes, *Rhabditophanes schneideri* (Bütschli) Goodey and *Diplogasteritus nudicapitatus* (Steiner) Paramonov, from treated sewage, could ingest the Coxackie and Echo viruses which are pathogenic in humans. Further, they demonstrated the viruses survived passage through the alimentary canal of the nematodes and while inside were protected against both routine (3–5 ppm) and excessive (100 ppm) chlorination even though the latter eventually killed the nematodes. In 1968 Jensen and Gilmour (6) reported the saprozoic nematodes, *Pristionchus lheriteiri* and *Panagrellus redivivus* (L.) Goodey transmitted the 514–S strain (Gilmour and Buthala) of phage to *Streptomyces griseus* (strain 3475 Waksman).

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MATERIALS AND METHODS

The nematode selected for the phage transmission studies was *Pristionchus lheriteiri* obtained from laboratory cultures maintained on a common soil bacterium. The chosen indicator bacterium was *Agrobacterium tumefasciens* (Strain 5–14 Deep), a strain maintained in laboratory culture. The phage (unnamed) was previously isolated from raw sewage at the Corvallis, Oregon (USA) treatment plant by Buangsuwon (2).

Four procedures were used to investigate relationships between nematodes and the phage. The first, included a series of manipulations to change the nematodes' food supply from a common soil bacterium to agar plate cultures of *A. tumefaciens*. This procedure is somewhat complicated and has been described in detail elsewhere (2). In the second step nematodes from phage-infected *A. tumefaciens* cultures were surface sterilized 20 min in a 20 ppm solution of chlorine (prepared by the Iodometric Method from a stock solution of sodium hypochlorite). Free chlorine was neutralized by adding an autoclaved solution of sodium thiosulphate ($Na_2S_2O_3$) (4). A third, involved the isolation and cultivation of the bacteriophage by the double-layer method (1). A fourth, was necessary to determine the phage content of the alimentary tract before defecation. This was done by chlorinating nematodes from phage plates, then crushing them with a

dissecting needle in sterile distilled water to release the phage particles.

Ingestion and survival of phage particles usually were determined by allowing the nematodes to feed in phage plates for 24 hr before rechlorination and then placing them upon newly-seeded *A. tumefaciens* plates. Procedures for determining phage density, longevity and viability were similar to those previously mentioned except the surface-sterilized nematodes obtained from the phage plates were transferred to nutrient agar plates containing 1,000 ppm tetracycline hydrochloride. The antibiotic prevented bacterial growth, eliminated the nematodes' food source, and prevented development of additional phage. At 3-hr intervals a sample of nematodes was removed and chlorinated. One-half of this sample was tested for phage passage by placing the nematodes upon newly-seeded agar plates of *A. tumefaciens*. The remainder of the nematodes were crushed in sterile distilled water and then plated by the double-layer agar method.

The number and extent of plaques (cleared zones) that developed on newly-seeded plates of *A. tumefaciens* were the criteria for estimation of phage density, survival and infectivity. All readings were taken after a 15–24 hr incubation period at 25 C.

RESULTS AND DISCUSSION

Pristionchus lheriteiri was a vector of an unnamed phage as indicated by plaque formation in newly seeded plates of *A. tumefaciens*. All nematode developmental stages except eggs or enclosed larvae ingested and defecated viable phage. These studies were, however, primarily concerned with phage associations of adult nematodes. Females uniformly acquired more phage faster and retained it longer than males and larvae perhaps in part reflecting their larger size and more aggressive feeding habits.

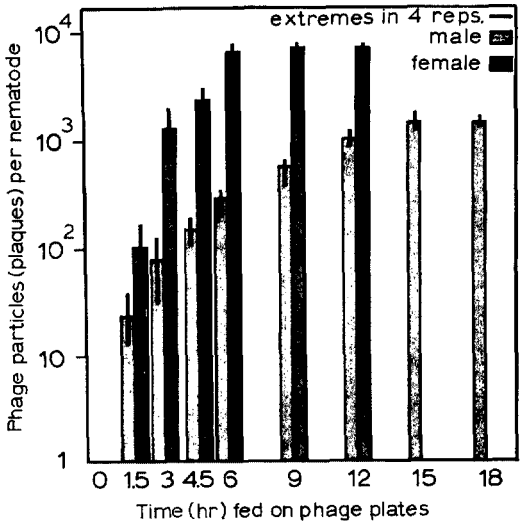

FIG. 1. Average plaque count of *Agrobacterium tumefaciens* phage voided by adult *Pristionchus lheritieri* (4 reps., 4 nemas per rep.) after various feeding periods.

Acquisition of the phage takes very little

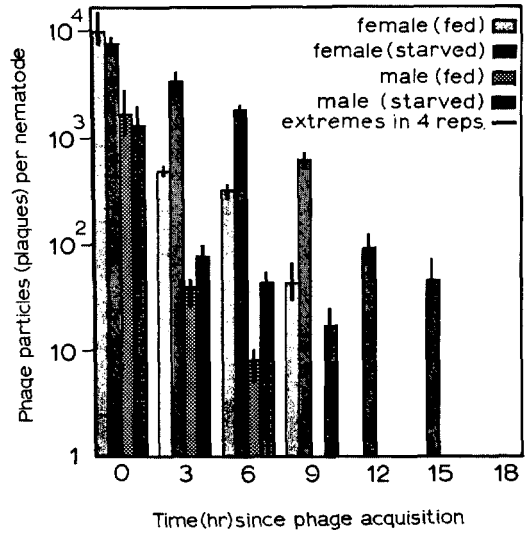

FIG. 2. Effects of feeding and starving upon the average number of *Agrobacterium tumefaciens* phage particles retained by adult *Pristionchus lheritieri* (4 reps., 4 nemas per rep.).

time as viable phage was eliminated after the minimum 1.5 hr feeding period (Fig. 1). Maximum phage accumulation in the female occurred after 12 hr of feeding and in the male after 15–18 hr of feeding, although there were no significant differences after 6 hr in the female and 12 hr in the male (Fig. 1). Nematodes deprived of food retained the phage 3–6 hr longer than those allowed to feed (Fig. 2). These data also indicate the female retains phage 3–6 hr longer than the male and that phage survival decreased rapidly with retention time.

Demonstration of transmission of a phage able to lyse cells of an important plant pathogenic bacterium is of potential importance in plant disease control. That phage can survive in and be defecated by nematodes leads to the hypothesis that the nematode may have an active function in the edaphic environment.

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