# Survival of Chlorophyceae Ingested by Saprozoic Nematodes[1]

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*Abstract:* The saprozoic nematode, *Pristionchus lheritieri* ingested cells of four species of unicellular Chlorophyceae (grass-green algae) including *Chlamydomonas reinhardi* and unidentified species of *Ankistrodesmus, Chlamydomonas* and *Scenedesmus.* Additional tests with *Ankistrodesmus* sp. and *Chlamydomonas* sp., indicated cells of *Ankistrodesmus* survived passage through the alimentary canal and were subsequently cultured, while viable cells of *Chlamydomonas* were only occasionally recovered.
*Key Words: Pristionchus lheritieri,* Ingestion, Survival, Chlorophyceae, Algae, *Chlamydomonas reinhardi, Chlamydomonas* sp., *Ankistrodesmus* sp., *Scenedesmus* sp.

In 1960, Chang, Berg and Clarke (1) investigated the role of pathogenic microorganisms in municipal water supplies and demonstrated that two species of saprozoic nematodes (*Cheilobus quadrilabiatus* and *Diplogaster nudicapitatus*) ingested a number of human pathogenic microorganisms which thus survived excessive chlorination treatments. Recently, studies by Jensen (3) showed that saprozoic nematodes (principally *P. lheritieri*) ingested various plant pathogenic bacteria and fungal spores which survived passage through the alimentary canal. Apparently, there has been little or no investigation of possible interrelationships of saprozoic nematodes and algae.

## MATERIALS AND METHODS

A saprozoic nematode, *Pristionchus lheritieri,* (Maupas) Paramonov, was cultured in Petri dishes on nutrient agar medium (3 g beef extract, 5 g peptone, 15 g agar in one liter distilled water). An unidentified *Pseudomonas* sp. bacterial contaminant present on the cuticle or in the intestine provided a food source. These cultures were easily maintained by transferring small plugs of agar containing nematodes to new plates every 6 or 7 days.

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Algae used in this study were *Chlamydomonas reinhardi,* Dangeard, unidentified species of *Ankistrodesmus, Chlamydomonas,* and *Scenedesmus.* The algae were cultured in glass jars which contained 100 or 150 ml of mineral culture media. Two such media were used: H. C. Bold's Culture Solution #8 (5) and Bristol's Solution as modified by H. C. Bold (6). Cultures were aerated by a small air pump which also reduced sedimentation and kept the nutrients mixed. Clear plastic covers on the jars permitted penetration of 225 ft-c surface intensity from fluorescent tubes suspended above the cultures. Photoperiods were 8 a.m. to 5 p.m. and 11:30 p.m. to 1:30 a.m. daily. New cultures were started every three weeks by aseptic transfer of a bit of inoculum to fresh media.

A semi-solid mineral medium was prepared by the addition of agar to Bold's Culture Solution. Petri dishes of this agar were inoculated with several drops of algal culture suspension. Then 50 to 60 nematodes were added and permitted to feed 4 to 5 days. The same photoperiods were used for these plates as were used for cultures of algae in liquid media. Whole, water-mounted nematodes from these plates were examined microscopically for ingested cells.

To determine viability of ingested algal cells, the nematodes were first surface-sterilized either by thorough washing in distilled water or by rinsing 20 min in 20 ppm (residual) chlorine solution and then examined

for algae adhering to the cuticle. Surface-sterilized nematodes were crushed with a dissecting needle and the intestinal contents were transferred to sterile agar and observed for growth of algae. Other surface-sterilized nematodes were placed on agar to test for viable algae voided in the feces.

Several experiments were conducted to determine the optimum time and concentration of chlorine lethal to species of *Ankistrodesmus* and *Chlamydomonas*. Similar data available for *P. lheritieri* (2) indicate exceptional tolerance to this biocide. A thick algal suspension was mixed with chlorine solutions to give the desired concentrations of residual chlorine ranging from 0.60 ppm to 100 ppm. Samples of these mixtures were taken at timed intervals (ranging from 2 to 60 min after adding the algae) and added to a sodium thiosulfate solution (final concentration = 1%) to neutralize the chlorine. A 2 ml sample of each treatment was plated on Bold's agar and incubated under the same conditions for the liquid algal cultures.

## Results

Microscopic examination of nematodes after 4 to 5 days feeding on algal cultures revealed that all four algae were ingested. Numbers of cells ingested varied from a few scattered throughout the intestine lumen to great concentrations packed in the anterior intestine near the esophagus or in the posterior portion near the cloaca or rectum. Photomicrographs were obtained of each of the four algae in nematode intestine (Fig. 1, A–D).

Two techniques used to check viability of ingested algal cells gave parallel results. Viable cells of *Ankistrodesmus* sp. were recovered from both the intestinal contents and feces of *P. lheritieri*, but viable cells of *Chlamydomonas* sp. rarely were recovered.

At least 30 min exposure to 40 ppm chlorine was necessary to completely kill cells of *Chlamydomonas* sp. which had clumped together in a palmelloid cyst. If aeration was adequate in the liquid algal culture, however, the cells remained single and were effectively killed by 20 ppm chlorine for 20 min. Preliminary work with *Ankistrodesmus* sp. indicated that exposure to 20 ppm for 15 min was lethal.

## Discussion

*P. lheritieri* can ingest cells of four unicellular Chlorophyceae: *C. reinhardi*, and unidentified species of *Ankistrodesmus*, *Chlamydomonas*, and *Scenedesmus*. Furthermore, it seems quite possible that the digestive enzymes or physical-chemical factors in the alimentary canal of *P. lheritieri* are selectively detrimental to algae. Our studies indicated *Ankistrodesmus* survived passage through the alimentary canal while *Chlamydomonas* rarely survived. Since there is no detailed information on digestive enzymes or other physical-chemical features in the alimentary canal of this microbivorous nematode (4), further investigation is clearly indicated. Another comparative study of toleration to these various factors by *Ankistrodesmus* and *Chlamydomonas* might well be enlarged to include *C. reinhardi*, *Scenedesmus*, other unicellular Chlorophyceae and possibly some of the blue-green algae. Our results suggest that *P. lheritieri* is a selective agent in the distribution of unicellular algae.

Investigations involving the chlorination of algae are only preliminary and thus no

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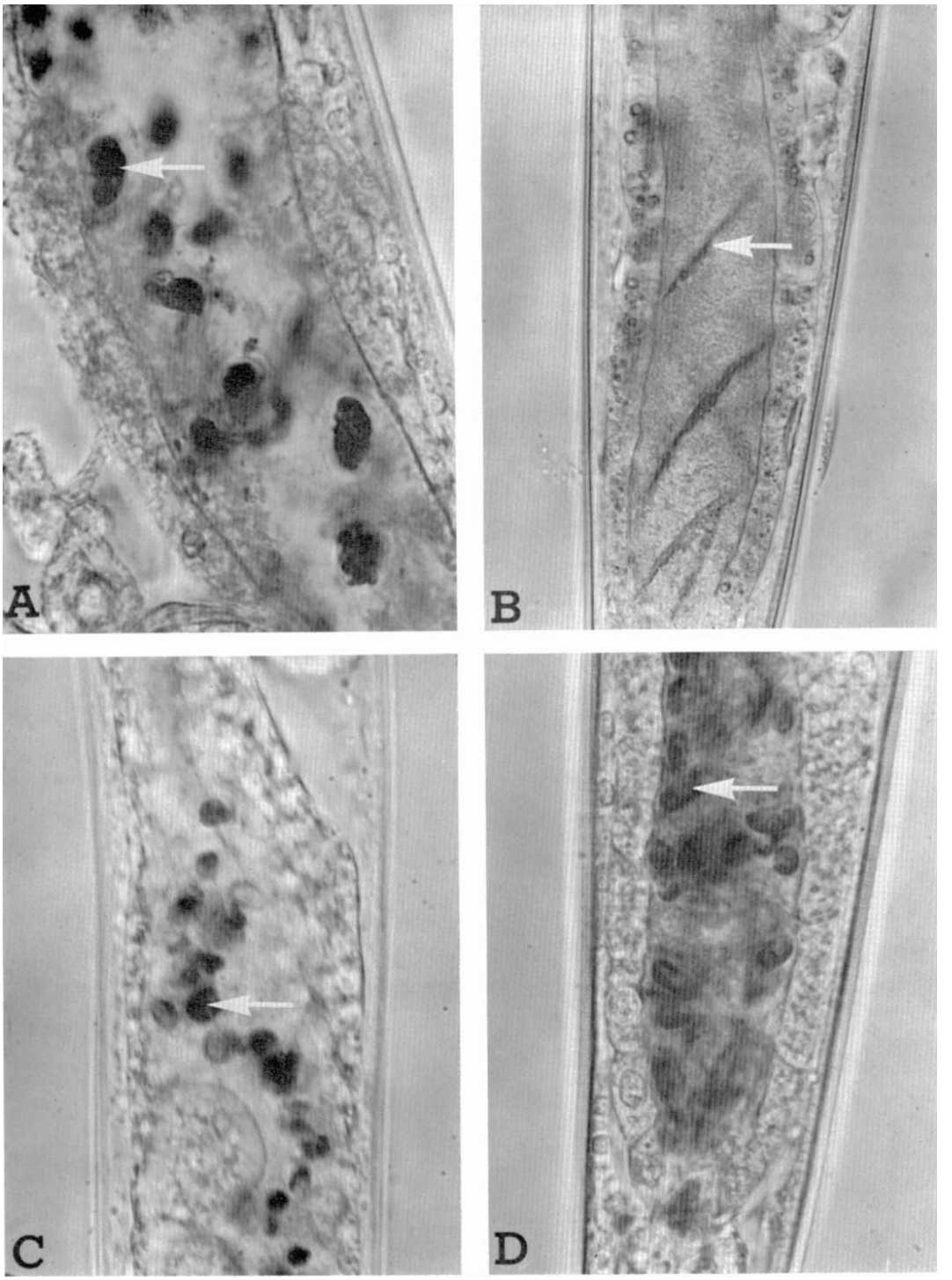
FIG. 1. Photomicrographs of *Pristionchus lheritieri* showing a section of the body with the intestinal tract containing various ingested algae (Note arrows). **A.** *Chlamydomonas* sp.; **B.** *Ankistrodesmus* sp.; **C.** *Chlamydomonas reinhardi*; **D.** *Scenedesmus* sp.


A
B
C
D

general conclusion can be reached. Previous work by Jensen (3) and results presented above indicate, however, that algal cells in the intestine of saprozoic nematodes would be unharmed by concentrations of chlorine which would be lethal to exposed cells. Hence, an alga highly tolerant of the digestive enzymes of *P. lheritieri* could survive during ingestion in an otherwise lethal environment, while an alga with low tolerance would not survive. Again, the need of further investigation in this direction is indicated.

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