Morphological and behavioral abnormalities in *Heterorhabditis heliothidis* (Rhabditida) associated with the bacterium, *Alcaligenes faecalis*

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SUMMARY

Cultures of the entomogenous nematode, *Heterorhabditis heliothidis* grown on artificial media, suddenly declined in productivity as a result of the bacterial contaminant. *Alcaligenes faecalis*. The contaminated cultures produced infective stage juveniles that exhibited neoplasms as well as other morphological and behavioral abnormalities. Some of these abnormalities could be induced by adding *A. faecalis* and a liquid filtrate of *A. faecalis* to monoxenic cultures of developing nematodes. These abnormalities of *H. heliothidis* are considered a result of teratogenic effects from the contaminating bacterium, *A. faecalis*.

RÉSUMÉ

Anomalies morphologiques et comportementales chez Heterorhabditis heliothidis *(Rhabditida) associé à la bactérie* Alcaligenes faecalis

Les élevages en milieu artificiel du nématode entomophile *Heterorhabditis heliothidis* peuvent voir leur productivité décliner brusquement sous l'action d'une bactérie contaminante, *Alcaligenes faecalis*. Les élevages ainsi contaminés contiennent des juvéniles infestants montrant des formations néoplasiques et diverses anomalies morphologiques et comportementales. Certaines de ces anomalies ont pu être induites par addition d'*A. faecalis*, ou d'un filtrat de cette bactérie, à des élevages monoxéniques du nématode. Ces anomalies de *H. heliothidis* sont donc considérées comme provenant de l'action tératogène de la bactérie contaminante *A. faecalis*.

A noted decline in monoxénic *in vitro* cultures of *Heterorhabditis heliothidis* (Khan, Brooks & Hirschmann) (Heterorhabditidae : Rhabditina) prompted an examination in attempts to determine the cause of the condition. The nematodes were being grown on an autoclaved pig kidney medium that had been previously inoculated with the symbiotic bacterium, *Xenorhabdus luminescens*. No metazoan, fungal or protozoan contaminants were noted but the presence of a foreign bacterium was demonstrated by plating out samples of the medium on nutrient agar; the result of this contaminated condition was the production of a certain proportion of infective juveniles which exhibited neoplastic growths as well as other morphological and behavioral abnormalities. The present paper describes these abnormal symptoms and relates their presence to the contaminating bacterium.

Materials and methods

Plate colonies made from the nematode *in vitro* medium consistently revealed the presence of a single foreign bacterium, which was submitted to the Microbial Disease Laboratory of the California Department of Health Services in Berkeley, California, for determination.

This bacterium was cultured in peptone water (1 % bacto peptone, 0.5 % NaCl in water) for 24-48 hours at 24°. Cells from this medium (0.2 cm) were placed directly on five sterile dog food agar slants (50 ml tubes) each containing a bixenic culture of *H. heliothidis* and *X. luminescens*. A cell free supernatant of the peptone water culture of the bacterium was obtained by centrifuging the medium in a clinical centrifuge at 3 000 rpm for 30 minutes, then passing the supernatant through a 0.25 µm Milipore filter. This cell free supernatant was also added to five dog food agar slants containing bixenic cultures of *H. heliothidis* and *X. luminescens*.

Results

Cultures of the foreign bacterium sent to the Microbial Disease Laboratory of the California Department of Health Services in Berkeley, California, were identified as *Alcaligenes faecalis* Castelloni & Chalmers (also known as *Alcaligenes odorans* Málek & Kazdová-Kovisková and *Pseudomonas odorans* Málek & Kazdová-Kovisková).

ABNORMAL SYMPTOMS

Original cultures of *H. heliothidis* that were contaminated with *A. faecalis* showed a range of morphological and behavioral abnormalities. Observations were made on the third stage infective juveniles which were better able to survive the contaminated conditions. In nature, this stage seeks out and enters the body cavity of insect

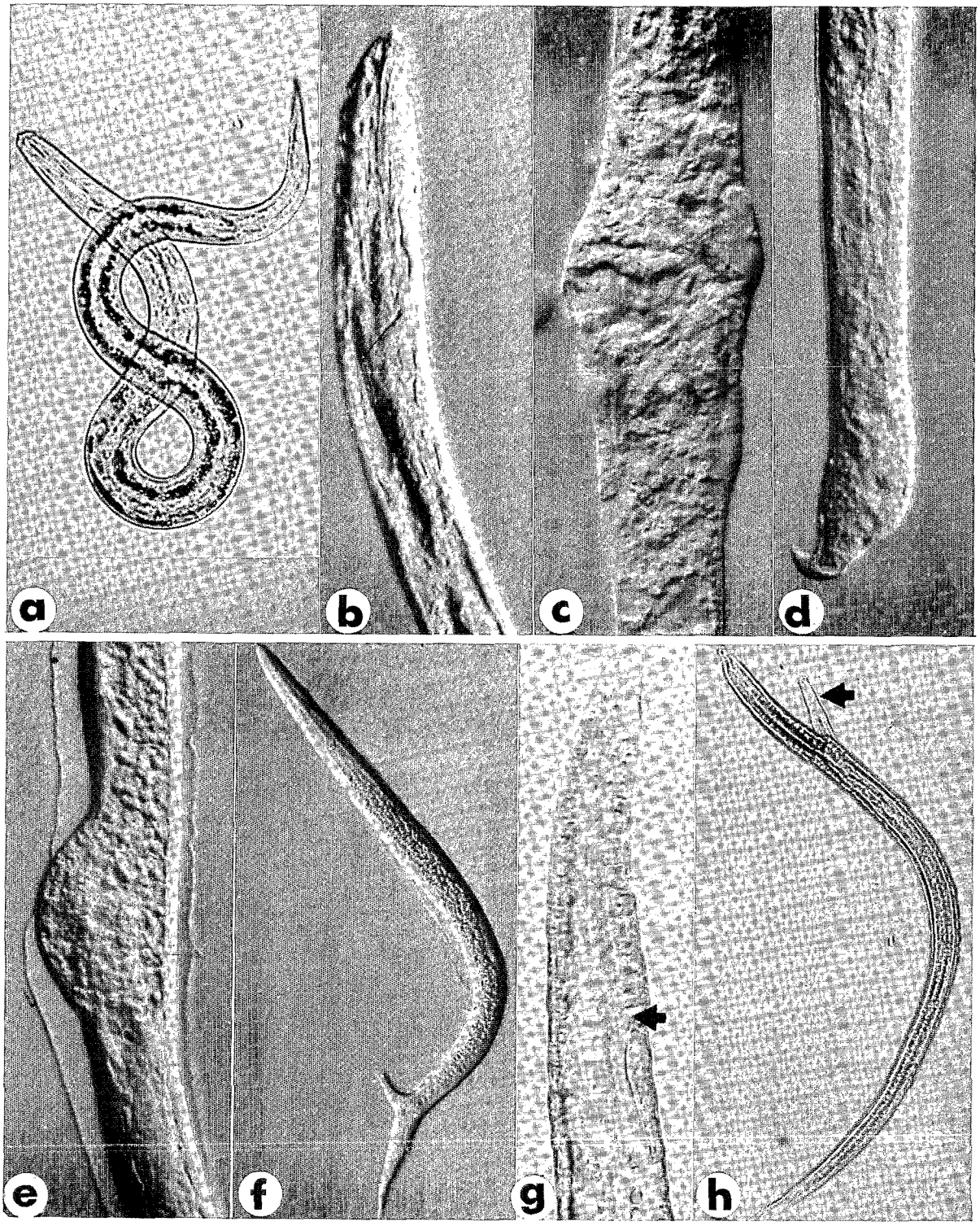

Fig. 1. Infective juveniles of *Heterorhabditis heliothidis* obtained from *A. faecalis* contaminated cultures. a. Nematode showing starved, coiled syndrome; b : Nematode showing abnormal development in neck region; c : Nematode showing midbody neoplasm; d : Nematode showing swollen condition in tail region; e : Nematode with neoplastic growth in tail region; f : Nematode exhibiting a dorsal posterior " horn "; g : Thickened second stage cuticle with enclosed third stage juvenile attempting to penetrate (arrow = head of third stage juvenile); h : Head of a third stage juvenile (arrow) emerging from the body wall of its thickened second stage " cuticle ".

hosts (Poinar, 1979). Approximately 50 % of these infective stages showed signs of starvation and inanition (e.g., hyaline body and intestinal cells containing few food reserves). These nematodes were quiescent and rested almost continuously in a single or double coiled position as if they were partially paralyzed (Fig. 1 a). This position is never assumed in normal infectives, which lie in a straight or slightly curved posture. From 1-5 % of the infective stages exhibited neoplastic abnormalities in the neck, midbody and posterior region (Fig. 1 b, c, d, e). Included in the latter category were two individuals (out of 5 000) which possessed a dorsal " horn " near the tail, giving the appearance of a forked tail (Fig. 1 f).

In one batch, approximately 40 % of the infectives of *H. heliothidis* exhibited abnormalities associated with the structure and molting of the second stage cuticle. This cuticle is normally thin and membranous and surrounds the infective stage after it leaves the dead insect. It eventually is lost as the nematode moves through the soil in search of insect hosts. In the contaminated cultures, the second stage cuticle did not consist of only cuticle but also of associated hypodermal cells which had become large and rigid. Thus the third stage infective juvenile formed inside the body wall of the second stage and frequently became trapped there, although some would manage to penetrate and emerge from the body wall (Fig. 1 g, h).

Experimental results

Infectives of *H. heliothidis* that exhibited the more common coiled starved syndrome were surface sterilized and placed on sterile dog food agar. They still carried their natural symbiont *(X. luminescens)* and most developed normally, producing normal infective stages. When colonies of *A. faecalis* were added to dog food cultures of *H. heliothidis* that had been inoculated with infective juveniles, the infectives developed into hermaphroditic females but eggs from these females developed into infectives, without going through an amphimictic generation as is customary for this species. These infective juveniles exhibited a high proportion of the coiled, starved syndrome (about 50 %). Those cultures that received the cell free filtrate of *A. faecalis* exhibited a lower rate of coiled, starved infectives (about 20 %). The more severe neoplastic abnormalities were not observed in the experimentally contaminated dog food cultures. Infectives of *H. heliothidis* in the control cultures of dog food tubes containing bixenic populations did not exhibit the coiled, starved syndrome.

Discussion

Morphological and behavioral abnormalities in an organism, as reported here, could be caused by either genetic or non-genetic factors. Genetic factors, as well as those resulting from mutagenic lesions, are distinguishable from non-genetic factors (such as teratogenic responses) by being transmissible to future generations (Manson, Zenick & Costlow, 1984). Since there was no evidence of the abnormalities being carried over in future generations in the experiments reported, it is concluded that the present modifications are due to an agent produced by *A. faecalis* which caused damage in the developing embryos resulting in neoplastic and other abnormalities in the infective stages. Most teratogens have specific mechanisms of action and can cause birth defects at doses below those causing maternal toxicity. Teratogens are known to cause non-genetic deformities in vertebrates as a result of affecting the supply of energy sources and substrates, inhibiting enzymes, altering membrane permeability, affecting osmolar imbalance and tissue elements or their biochemical products (Manson, Zenick & Costlow, 1984). There may be multiple sites of attack in the present case since there is the coiled, starved syndrome which is associated with an interference in the energy supply and the neoplastic malformations which could result from altered cell membranes. Further studies are required to elucidate these points.

This bacterium has been recovered from infective juveniles of the related insect parasitic nematode, *Neoaplectana carpocapsae*, in Czechoslovakia (Málek, Radochová & Lysenko, 1963), however no abnormalities of the nematode were noted.

The present report represents the first known instance of neoplasms in nematodes resulting from apparent teratogenic effects of a foreign bacterium in the environment.

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