INVOLVEMENT OF LARVICIDAL TOXINS IN PATHOGENESIS OF INSECT PARASITISM WITH THE RHABDITOID NEMATODES, STEINERNEMA FELTIAE AND HETERORHABDITIS BACTERIOPHORA

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The insect-parasitic rhabditoid nematodes, Steinernema feltiae and Heterorhabditis bacteriophora, released a compound/s/ toxic to larvae of the greater wax moth, Galleria mellonella, that caused paralysis and death of the insect. Larvicidal substances appeared in wax moth larvae during parasitism and after inoculation with the primary form of the bacterial associates of the nematodes. The nematode S. feltiae and its associate, Xenorhabdus nematophilus, excreted much less toxic activity within larval body than H. bacteriophora. The secondary form of Xenorhabdus did not produce toxin in parasitized larvae, but X. luminescens, the bacterium associated with H. bacteriophora, released detectable titer of toxin activity in broth cultures. Both nematode toxins were sensitive to heat and produced a specific type of proteolytic activity. Preliminary identification of the compounds responsible for larval toxicity revealed similarities to immune inhibitors produced by some bacterial pathogens of insects.

KEY-WORDS: Steinernema feltiae, Heterorhabditis bacteriophora, Xenorhabdus, nematode toxin, immune inhibitor.

The entomogenous rhabditoid nematodes, Steinernema feltiae (= Neoplectana carpopodisae) and Heterorhabditis bacteriophora, have a biologically intricate process of pathogenesis. They harbour Gram-negative asporous, rod-shaped bacteria of the genus Xenorhabdus (Poinar, 1966; Akhurst, 1982). The non-feeding infective stages carry these symbiotic bacteria monoxenically within the intestine. S. feltiae contains the bacterium X. nematophilus; X. luminescens is associated with H. bacteriophora (Poinar, 1966; Milstead, 1979). After entering the host insect, the invasive stage of the nematode penetrates the haemocoel and releases its bacterial associates. The bacteria proliferate, kill the insect, and establish suitable conditions for reproduction of the nematode by providing nutrients and suppressing the growth of contaminating microorganisms (Poinar & Thomas, 1966).

Each bacterial associate occurs in 2 biochemically similar dimorphic forms, designated the primary and the secondary form (Akhurst, 1980). The forms differ morphologically on Tergitol-7-agar with triphenyltetrazolium chloride and differ in their abilities to support nematode growth and reproduction. Nematodes reproduce more rapidly and more prolifically with the primary form (Bedding, 1981). This form is unstable and occurs in mature infective-stage nematodes and in the insect haemocoel after release. The secondary
form is usually stable, is occasionally produced within the host cadaver, and inevitably occurs on artificial media. The role of the secondary form in mutualism has not been determined.

Many researches have investigated the rhabditoid nematodes of the families *Steinernematidae* and *Heterorhabditidae* as potential agents for biological control of insect pests (Schmieg, 1963; Sandner & Stanuszek, 1971; Triggiani & Poinar, 1976; Poinar, 1979; Kaya & Hara, 1980; Poinar et al., 1983; Finney & Bennett, 1983; 1984; Morris, 1985). Others have examined mutualistic associations between entomophagous nematodes and intestinal bacteria (Poinar, 1966; Poinar & Thomas, 1966; Poinar et al., 1977; Thomas & Poinar, 1979; Akhurst, 1980; 1982; and 1983). Much less research has considered the role of toxic substance(s) produced either by the nematodes or their bacterial associates in entomopathological processes. Boemare et al. (1982) stated that an entomocidal factor, not dependent on mutualistic bacteria, appears in axenic *G. mellonella* parasitized by axenic *S. feltiae* during the 4th larval stage of nematodes developing in the host. Similarly, Burman (1982) found a toxin produced periodically in artificial liquid media during life cycle of axenic *S. feltiae*. Maximal toxic activity occurred in cultures containing a high percentage of adult nematodes. It disappeared after hatching of juveniles and then reappeared with the development of the 3rd- and 4th-stage nematodes. The activity and behaviour of the greater wax moth inoculated with the crude toxin were rapidly affected. Moreover, Boemare et al. (1982) suggested that the toxin of *S. feltiae* may interfere with the antibacterial defence of the host insect.

This paper presents further observations on the toxic compounds to assess the involvement of this larvicidal factor produced by nematode-bacterial complex and by the primary form of the bacteria in pathogenesis of insects parasitized by *S. feltiae* and *H. bacteriophora*.

MATERIALS AND METHODS

NEMATODES AND INSECT HOST

The nematodes examined in this study are indigenous Polish strains isolated by Dr. H. Skrzypek from soil at Lublin using the Galleria traps technique of Bedding & Akhurst (1975).

Late seventh instars of the greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae), were used to study gross pathology and histopathology and for toxicological bioassays. Infective stages of *S. feltiae* and *H. bacteriophora* were collected from parasitized larvae of the greater wax moth. Juveniles were stored at 6 °C in a thin layer of 0.004 % formaldehyde solution which was aerated at two-week intervals.

SELECTION OF PRIMARY AND SECONDARY FORM OF XENORHABDUS

Pure cultures of the primary form were obtained according to Akhurst (1980) by crushing surface-sterilized juveniles in a tissue homogenizer. The homogenates were streaked onto Tergitol-7-agar (Poinar & Thomas, 1978) and incubated at 23 °C until colonies of the primary form could be differentiated morphologically. The irregular, green colonies that were surrounded by decolorized zones after 7-12 days were classified into group II (Akhurst, 1983) of the primary bacteria.

The secondary forms were obtained from the plasma of larvae parasitized by nematodes. The red colonies on Tergitol-7-agar were subcultured several times to ensure pure