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Pathogenic effect of entomopathogenic nematode–bacterium complexes on terrestrial isopods

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Abstract

In this study, we evaluated the effect of entomopathogenic nematodes (EPNs) *Steinernema carpocapsae*, *Steinernema feltiae* and *Heterorhabditis bacteriophora*, symbiotically associated with bacteria of the genera *Xenorhabdus* or *Photorhabdus*, on the survival of eight terrestrial isopod species. The EPN species *S. carpocapsae* and *H. bacteriophora* reduced the survival of six isopod species while *S. feltiae* reduced survival for two species. Two terrestrial isopod species tested (*Armadillidium vulgare* and *Armadillo officinalis*) were found not to be affected by treatment with EPNs while the six other isopod species showed survival reduction with at least one EPN species. By using aposymbiotic *S. carpocapsae* (i.e. without *Xenorhabdus* symbionts), we showed that nematodes can be isopod pathogens on their own. Nevertheless, symbiotic nematodes were more pathogenic for isopods than aposymbiotic ones showing that bacteria acted synergistically with their nematodes to kill isopods. By direct injection of entomopathogenic bacteria into isopod hemolymph, we showed that bacteria had a pathogenic effect on terrestrial isopods even if they appeared unable to multiply within isopod hemolymphs. A developmental study of EPNs in isopods showed that two of them (*S. carpocapsae* and *H. bacteriophora*) were able to develop while *S. feltiae* could not. No EPN species were able to produce offspring emerging from isopods. We conclude that EPN and their bacteria can be pathogens for terrestrial isopods but that such hosts represent a reproductive dead-end for them. Thus, terrestrial isopods appear not to be alternative hosts for EPN populations maintained in the absence of insects.

Keywords: *Steinernema carpocapsae*; *Steinernema feltiae*; *Heterorhabditis bacteriophora*; *Xenorhabdus nematophila*; *Xenorhabdus bovienii*; *Photorhabdus luminescens*; Entomopathogenic nematodes; Entomopathogenic bacteria; Terrestrial isopods; Pathogenicity; Symbiosis

1. Introduction

Entomopathogenic nematodes (EPNs) in Steinernematidae and Heterorhabditidae are insect pathogens symbiotically associated with Enterobacteriacea from the genera *Xenorhabdus* and *Photorhabdus*, respectively. The infective juveniles (IJs) which live in the soil are able to penetrate into various insects (Laumond et al., 1979; Poinar, 1979). After entering the host, IJs release their bacterial symbionts which multiply in the insect hemolymph causing septicemia. In the host cadaver, the nematode adults reproduce for several generations leading to the production of offspring (IJs) which emerge from the insect in the soil (Poinar and Thomas, 1966; Sicard et al., 2004).

Experimental and field studies have shown that these nematode–bacterium complexes attack a wide range of insects and appear to be mainly restricted to this group of invertebrates. Nevertheless, possible penetration, development and multiplication within non-insect hosts have also been reported suggesting these EPNs and their symbiotic bacteria could have consequences on soil invertebrates other than insects (Jaworska, 1993; Poinar, 1989). As terrestrial isopods are soil inhabitants acting mainly as scavengers feeding on decaying plant and animal material, frequent interactions should occur between them and entomopathogenic nematode–bacterium complexes. If

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isopods are susceptible to these complexes, we could postulate that the latter can have a non-negligible impact on terrestrial isopods populations. Previous experimental studies have shown that isopods from the genera *Armadillidium* and *Porcellio* could be experimentally infected and killed by some EPN species (Jaworska, 1994; Poinar, 1989; Poinar and Paff, 1985) but that other EPN species were non-pathogenic for them (Eng et al., 2005). For ecologists, these results indicate that some isopod species could constitute alternative hosts for EPNs and provide nematodes and bacteria with interesting niches where they could maintain when insects are scarce. In a pest control perspective, the susceptibility of terrestrial isopods towards EPNs suggests that the latter may constitute a non-chemical alternative for controlling terrestrial isopods which can destroy seedlings of sweet peas and a number of flowering plants especially in a greenhouse environment (Jaworska, 1991; Poinar and Paff, 1985). In this study, we performed experimental infection of isopods belonging to eight species with three different entomopathogenic nematode–bacterium complexes. This approach led us to define which isopods were sensitive or resistant to entomopathogenic complexes and which EPN complexes were more pathogens of isopods.

In order to investigate if bacteria (*Xenorhabdus* and *Photorhabdus*) are solely pathogenic against isopods, we injected them directly into hemolymph of two terrestrial isopod species, *Armadillidium vulgare* and *Porcellionides pruinosus*, and mortality was recorded. Moreover, for the nematode–bacterium complex formed by *Steinernema carpocapsae* and *Xenorhabdus nematophila*, we compared the mortality of isopods by nematodes that did not carry *X. nematophila* (i.e. aposymbiotic nematodes) and the mortality caused by nematodes carrying *X. nematophila* (i.e. symbiotic nematodes). Such comparisons have been previously performed to investigate the role of each partner in the pathogenic effect of nematode–bacterium complexes towards one host (Ehlers et al., 1997; Han and Ehlers, 2000; Sicard et al., 2003; Mitani et al., 2004). The possible maintenance of entomopathogenic complexes in terrestrial isopod populations was assessed by evaluating in different terrestrial isopod species: (i) the ability of IJs to develop into adults within isopod cadavers, (ii) the ability of entomopathogenic bacteria to multiply in host hemolymphs and (iii) the ability of nematodes to produce new IJs emerging from isopod cadavers.

2. Materials and methods

2.1. Isopod maintenance

The eight species of isopods were reared in the laboratory at 22 ± 2 °C on moistened potting mix derived from peat from sphagnum moss (pH 6.4 and conductivity = 50.0 mS/m). The food source was dead leaves and carrot slices. The species name and original location of the different isopod species are summarized in Table 1.

Table 1
Isopod species used in the study and their geographical origin

Isopod species	Geographical origin (country, location)
Armadillidium vulgare	France, Angoulême
Armadillo officinalis	Tunisia, Tunis
Atlantoscia floridana	Brazil, Porto Alegre
Chaetophiloscia elongata	France, Celles sur Belle
Cylisticus convexus	France, Villedaigne
Oniscus asellus	UK, Edinburgh
Porcellio dilatatus	France, Rom
Porcellionides pruinosus	France, Nevers

2.2. Nematode stocks

The three EPNs used in this study belong to three different species: *S. carpocapsae* associated with *X. nematophila* F1 sampled in Plougastel (France), *Steinernema feltiae* associated with *Xenorhabdus bovienii* FR45 sampled in Aigues-Mortes (France) and *Heterorhabditis bacteriophora* associated with *Photorhabdus luminescens* subsp. *laumondii* FR42 sampled in Lansargues (France). These three EPN species were established in laboratory culture as soon as they were sampled by successive experimental infections on the last instar of *Galleria mellonella* (Lepidoptera: Galleridae). IJs used in experimental infection were all freshly emerged (less than 10 days) from *G. mellonella*. Aposymbiotic nematodes of *S. carpocapsae* were obtained by disinfecting the surface of the nematodes' eggs by crushing 40 mature females in sterile Ringer solution (NaCl 0.9% w/vol) with sodium hypochloride (10% w/vol) and incubating them in this solution for 18 min. The disinfected eggs were then rinsed twice with sterile Ringer solution and transferred into "liver-agar" plates (Sicard et al., 2003) for incubation at 24 °C. Three weeks later, axenic IJs were obtained from these plates, which were available for further experimentation. Sterility of the IJs was assessed by inoculating nutrient broth tubes with samples from the liver plates. Secondly, aposymbiotic nematodes were produced by infecting insects with axenic IJs. The IJs emerging from these infestations were not axenic as they came from insect cadavers that contain their own microflora. But these nematodes were considered aposymbiotic as they grew within the insects without *X. nematophila*.

2.3. Treatment of isopods with EPNs

In order to evaluate the effect of EPNs on female and male isopods, the different treatments with symbiotic nematodes were performed separately with 10 females and 10 males for all isopod species. Nevertheless, as asymbiotic IJs were difficult to produce, treatments with aposymbiotic *S. carpocapsae* were performed only for 10 females of *Atlantoscia floridana*, *Porcellio dilatatus*, *P. pruinosus* and *Oniscus asellus*. All treatments were conducted in 2-cm-deep, 9-cm-diameter glass Petri dishes filled to a depth of 1 cm with potting mix of the same