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Effects of an entomopathogen nematode on the immune response of the insect pest red palm weevil: Focus on the host antimicrobial response

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ABSTRACT

Relationships between parasites and hosts can be drastic, depending on the balance between parasite strategies and the efficiency of the host immune response. In the case of entomopathogenic nematodes and their insect hosts, we must also consider the role of bacterial symbionts, as the interaction among them is tripartite and each component plays a critical role in death or survival. We analyzed the effects induced by the nematode–bacteria complex *Steinernema carpocapsae*, against red palm weevil (RPW) larvae, *Rhynchophorus ferrugineus*. We examined the antimicrobial response of the insect when in the presence of nematocomplexes or of its symbionts, *Xenorhabdus nematophila*. In detail, we investigated the potential interference of live and dead *S. carpocapsae*, their isolated cuticles, live or dead bacterial symbionts and their lipopolysaccharides, on the synthesis and activity of host antimicrobial peptides. Our data indicate that both live nematodes and live bacterial symbionts are able to depress the host antimicrobial response. When nematodes or symbionts were killed, they lacked inhibitory properties, as detected by the presence of antimicrobial peptides (AMPs) in the host hemolymph and by assays of antimicrobial activity. Moreover, we isolated *S. carpocapsae* cuticles; when cuticles were injected into hosts they revealed evasive properties because they were not immunogenic and were not recognized by the host immune system. We observed that weevil AMPs did not damage *X. nematophila*, and the lipopolysaccharides purified from symbionts seemed to be non-immunogenic. We believe that our data provide more information on the biology of entomopathogenic nematodes, in particular concerning their role and the activity mediated by symbionts in the relationship with insect hosts.

1. Introduction

The insect parasitic nematode Steinernema carpocapsae (Rhabditidae, Steinernematidae) forms nematocomplexes associated with the mutualistic bacterium *Xenorhabdus nematophila* (Enterobacteriaceae, Xenorhabdus). These obligate parasites, also called entomopathogenic nematodes (EPNs), are natural regulators of insect populations, thus they are applied as biological control agents to suppress pest populations (Lacey and Georgis, 2012; Kaya and Gaugler, 1993). EPNs are able to reach and actively penetrate their targets; once inside the insect, because of unidentified signals, worms release their symbiotic bacteria into the host hemolymph, which are responsible for the death of the insect targets (Li et al., 2007; Eleftherianos et al., 2010). The life cycle of EPNs occurs through different temporal stages, each of which involves different biological events. Immediately after entry into the insect target, the nematode at the third juvenile stage (IJ3) is not recognized and thus eludes host immune-surveillance (short phase), then the nematode releases the bacterial symbionts responsible for drastic immune depression and consequent septicemic death of the host (medium and long phase). In the final phase, EPNs reproduce in the host corpse and offspring leave the insect in search for new targets. Fig. 1 summarizes the EPN life cycle correlated to the evasion/depression of host immune system (Wang and Gaugler, 1998; Brivio et al., 2002; Brivio et al., 2005; Brivio et al., 2010). As referred to above, nematocomplexes need larval corpses to complete their life cycle. For this reason, *Xenorhabdus* spp. symbionts perform a degradation process by converting larval tissues into biomass suitable for bacterial proliferation and nematode reproduction.

Many studies have suggested that the effectiveness of the nematode–bacteria complex is mostly due to the symbiotic bacteria, responsible for the mortality of the insect target. Several

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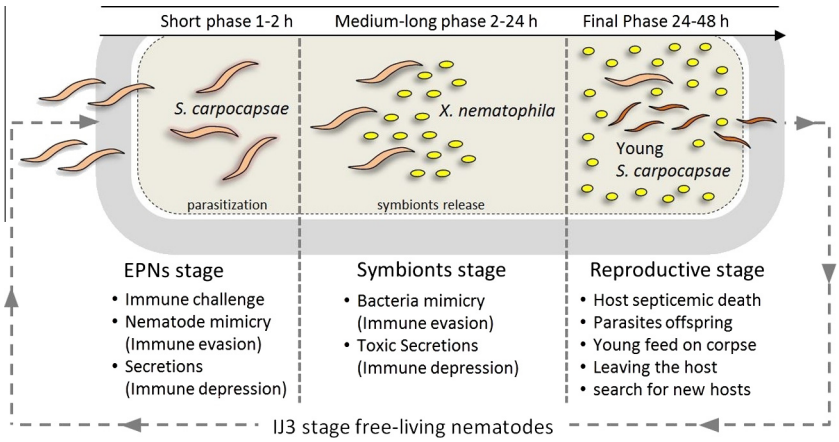

Fig. 1. The drawing outlines the life cycle of nematocomplexes (*Steinernema carpocapsae*/symbionts) inside the host.

authors have attributed the activity to the nematode itself, not only acting as a carrier, but also impairing the early immunological response of the host (Dunphy and Webster, 1987; Brivio et al., 2004; Ciche et al., 2005; Goodrich-Blair, 2007; Herbert and Goodrich-Blair, 2007; Mastore and Brivio, 2008; Tita et al., 2008; Dillman and Sternberg, 2012). As described by Peña et al. (2015) in *Drosophila* models, a contribution to the mortality of the host is provided by the nematode itself in its axenic form.

Insects react quickly against non-self, triggering effector processes leading to pathogen elimination from the hemolymph. Insect immune defense involves the interaction of pathogen-associated molecular patterns and pattern-recognition receptors (PAMPs and PRRs) and are mediated by cells and/or molecules involved in the recognition and neutralization of foreign substances (Gupta, 1991; Medzhitov and Janeway, 2002; McGuinness et al., 2003; Strand, 2008; Fors et al., 2014). Various active compounds present in the insect hemolymph, such as sugar-binding proteins, lectins, antibacterial peptides and enzymatic systems as the proPO system (prophenoloxidase–phenoloxidase) mediate the host humoral responses (Götz and Boman, 1985; Hoffmann et al., 1996; Söderhall and Cerenius, 1998). Some reactions are very responsive and prompt, such as activation of the proPO system (Leclerc and Reichhart, 2004), while other defense processes are active hours after infection. The latter category includes the activation of Toll and IMD pathways; these mechanisms stimulate fat body cells to synthesize and release antimicrobial peptides (AMPs) responsible for bacterial clearance (Zasloff, 2002; Nappi et al., 2004; Strand, 2008; Söderhall, 2010; Valanne et al., 2011; Mastore et al., 2014b).

Antimicrobial peptides are gene-encoded molecules that act as a first line of defense in innate immunity, ensuring the protection of the organism against environmental pathogens. AMPs possess various structural conformations, but most of them display hydrophobic and cationic properties with an amphipathic structure, and they commonly have a molecular mass below 20 kDa. Despite their structural variety, all natural AMPs are able to affect a large number of microorganisms *in vitro* (Bulet and Stöcklin, 2005) through the formation of channels in the bacterial wall; these channels increase membrane permeability, culminating in leakage of the cytoplasm (Hancock, 2001).

Various EPN evasive strategies have been described. Some of them are aimed at damaging host immune components by proteolytic secretions, as described for *S. carpocapsae* (Ribeiro et al., 1999; Balasubramanian et al., 2010; Toubarro et al., 2013). Different strategies are carried out by lipids or proteins of the body surface of *S. feltiae* or *S. glaseri*; these compounds are responsible for unrecognition and for interference with host immunological defenses (Wang and Gaugler, 1998; Brivio et al., 2004, 2006; Mastore and Brivio, 2008).

When *Xenorhabdus* spp. are released into the host hemocoel, they seem to be unrecognized and thus overcomes the host immune response; symbiont toxins affect the physiology of the insect, leading to general immunodeficiency (Stock and Goodrich-Blair, 2008; Dillman et al., 2012). Several studies have shown that *X. nematophila* interferes with bacterial clearance processes (Vallet-Gely et al., 2008; Crawford et al., 2012). Bacterial secretions seem to inhibit eicosanoid pathways, thereby impairing nodulation, phagocytosis and indirectly AMP synthesis, although in this case the exact mechanism of gene down-regulation remains to be elucidated (Ji and Kim, 2004; Park and Stanley, 2006; Aymeric et al., 2010; Hwang et al., 2013; Eom et al., 2014). Moreover, some *Xenorhabdus* strains seem to be insusceptible to the action of host immunocompetent factors (Duvic et al., 2012). In spite of many interesting data, deep knowledge of immune depression mechanisms induced by nematocomplexes is still missing, in particular regarding the effects of the nematode and its symbionts against the antimicrobial response of insects.

In a previous study, we isolated and partially purified a pool of antimicrobial peptides after immune challenge of the red palm weevil (RPW), *Rhynchophorus ferrugineus* (Mastore et al., 2014b) an important insect pest (Fiaboe et al., 2012); antimicrobial activity was evaluated against Gram negative and Gram positive bacteria strains. A 2D-PAGE pattern of partially purified host hemolymph showed the presence of almost 17 overexpressed or newly synthesized cationic peptides in the range from 4 to 30 kDa. Considering our results, in this work, we analyzed the effects induced by the infection of *S. carpocapsae* on the antimicrobial activity of the palm pest RPW. We studied the interference of dead or live nematodes on AMP synthesis in host larvae and we applied the same experimental strategy either to isolated *X. nematophila* or to their secretion products. We observed that live or dead *S. carpocapsae* are not immunogenic to RPW larvae. Then, to verify their roles in the down-regulation of antimicrobial peptide activity, we carried out double infections with *S. carpocapsae* and with a mixture of exogenous bacteria (*E. coli*/*B. subtilis*). The results indicate that live nematodes and their symbionts are able to suppress the AMP response in larvae. However, dead nematodes seem to lack these inhibitory properties, as we observed significant antimicrobial activity against *E. coli*. In addition, we assessed the effect of *S. carpocapsae* on the host antimicrobial response by means of sequential infections, combining parasitization with a second infection with exogenous bacteria. The presence of bacteria