Beneficial Nematodes: What is the Art of the Possible?

John M. Webster

There are many species and strains of Steinernema and Heterorhabditis and of their respective symbionts, Xenorhabdus and Photorhabdus. Selective use of the most appropriate nematode species/strain and/or selective utilization of the differing characters of these species and strains can temporarily decrease target insect populations. Genetic improvement and the exchange of bacterial symbionts may lead to improved biological control of insects. Enormous advances have occurred in the in vitro, mass production of entomopathogenic nematodes, utilizing industrial fermentation technology, which has enabled the production of the very large numbers of nematodes required for field applications. As well, improved storage, transport and application methods for the nematodes have ensured more consistent insect control. Nevertheless, understanding of the biology of Steinernema and Heterorhabditis in the soil is very incomplete. The attraction of Steinernema spp. to their target hosts is complex, and secretions of plants and the microflora, in particular, appear to change the nematode’s behaviour.

The beneficial characteristics of this nematode–bacterium complex to agroforestry are not limited to that of controlling insects. Many secondary metabolites produced by the bacterial symbionts have significant bioactive properties. Some of these compounds are antymycotic (e.g., xenocoumacins, xenorhabdins, nematophin), antibiotic (e.g., xenorhoxides, indoles), nematicidal (e.g., stilbenes) and insecticidal. The effective field use of some of these substances or of their derivatives can now be realized, and may play a key role when integrated in pest management strategies in the agroforestry industries.

Key words: Steinernema, Heterorhabditis, symbiosis, Xenorhabdus, Photorhabdus, biological control, nematode behaviour, secondary metabolites.

Coevolution of entomopathogenic nematodes and their insect hosts has resulted in a relatively stable biological relationship, a worldwide distribution of these nematodes and a wide range of host species. The intensive research focus in recent years has aimed at understanding and modifying this relationship in order to increase the number of insects killed by the nematode so as to enhance agro-forestry crop yields. However, only a few of the factors involved in this complex biological association of the entomopathogenic nematodes, their bacterial symbionts and insect hosts are understood. Basically, we are seeking to improve agro-forestry crop yields to our advantage what millions of years of evolution has perfected to a balanced advantage for the populations of nematodes and insects. Is this possible?

Biological species survival could be said to be based on adequate nutrition, shelter from adverse environments and predators and on successful reproduction. In this context, the biolog-

1 Department of Biological Sciences, Simon Fraser University, Burnaby, Vancouver, Canada.
cal success of *Steinernema* spp. and *Heterorhabditis* spp. is due to their symbiotic association with the bacteria, *Xerorhabdus* spp. and *Photorhabdus* spp., respectively.

A great asset in exploring this biological symbiosis is the species diversity among the nematode and bacterial symbionts. The differing host preferences, temperature effectiveness and virulence of the respective symbionts may make it possible to select the best nematode–bacterium combination to apply against a particular exotic or indigenous insect pest. As well, there is intraspecific variation among different geographic strains of the nematodes and bacteria. Both these bacterial genera typically have primary and secondary forms, and in some strains of *Photorhabdus* spp. a proportion of the bacterial cells have small colony variants that differ in their properties from the respective primary and secondary forms (Fig. 1; Table 1). For example, the small colony variant of *P. luminescens* strain MD achieves 100% insect mortality, but does so more slowly than does the primary form (17) (Fig. 2). Several researchers have explored the attributes and genetics of some of the species and strains of entomopathogenic nematodes (12, 14).

### Table 1. Characteristics of Vp (primary form) and Vsm (small colony variant) of *Photorhabdus luminescens* strain MD (after Hu et al., 1998).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Vp</th>
<th>Vsm</th>
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<tbody>
<tr>
<td>Cell size (um)</td>
<td>5.0 x 1.3 (3.8 - 8.0 x 1.0 - 1.8)</td>
<td>2.1 x 0.9 (1.5 - 3.0 x 0.8 - 1.0)</td>
</tr>
<tr>
<td>Proteinaceous granules</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Colony colour on TSA</td>
<td>Yellowish</td>
<td>Light grey</td>
</tr>
<tr>
<td>Colony size and form on TSAD</td>
<td>Large; dark green centre with radial stripes</td>
<td>Small; light green and homogenous</td>
</tr>
<tr>
<td>Colony adhesion</td>
<td>Strong</td>
<td>None</td>
</tr>
<tr>
<td>Bromothymol blue dye</td>
<td>Strong</td>
<td>Weak</td>
</tr>
<tr>
<td>Antibiotic production</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Luminescence</td>
<td>Strong</td>
<td>Weak</td>
</tr>
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</table>

- **Fig. 1** The proportion of primary form (Vp) and small-colony variant (Vsm) of *Photorhabdus luminescens* in a random sample of *Heterorhabditis* sp. infective juveniles. (K. Hu, pers. comm.)
- **Fig. 2** Percentage mortality of *Galleria mellonella* larvae infected with cells from either the primary form (Vp) or the small-colony variant (Vsm) of *Photorhabdus luminescens* at 200 cells/larva (after Hu & Webster, 1998).
A "HIGH ENERGY" DIET

BACTERIA
• Rapid growth
• Many generations

NEMATODES
• Mature & reproduce
• Two + generations

THE "COMPETITION"
• Alien bacteria
• Alien fungi
• Alien nematodes

Fig. 3 Diagramatic representation of the competing components for the “high energy” tissues in a Galleria mellonella cadaver infected by entomopathogenic nematodes.

and their bacteria (8) with a view to genetic improvement of marketable populations of a nematode species for the biological control of insects. We should recognize, however, that the subsequent nematode–bacterium population is probably biologically less stable, and may lose its virulence, change its host specificity or acquire properties that adversely effect its ability to be mass produced.

There have been major advances in our ability to mass produce the very large numbers of entomopathogenic nematodes that are needed for inundative applications against insect pests. In providing the high energy diet required for the developing nematodes (Fig. 3) we have advanced from culturing millions of Steinernema spp. on a dog food based medium (16) to their mass production in giant, fermentors (11). This has enabled customized formulations and large scale field applications at an economically acceptable cost (3, 13).

It is in the area of soil ecology that much research is needed. Although there is substantial evidence that the infective juveniles (IJs) of the nematodes can be transported from production to application sites without loss of virulence and that their field application can be achieved with a high degree of effective coverage (15) the level of insect mortality is often unpredictable. It is not clearly understood where and in what state the IJs survive in the soil or for how long a significant percentage of them retain their virulence. The IJs apparently survive better in some soils than in others, but whether this is due only to physio–chemical factors of the soil (24) or to the prevalence of parasites and predators of these entomopathogenic nematodes (2) is uncertain. As well, density-dependent factors within host cadavers influence the overall population dynamics of the nematodes (22).
Fig. 4 Change in the chemotaxis index (Cl) of *Steinernema feltiae* in response to cues, offered individually or in combination, by (a) unsterilized and (b) surface sterilized, *Galleria mellonella* larvae and root seedlings over 2 hrs (E. Hui, pers. comm.)

KLINGLER (19) showed that soil living, plant parasitic nematodes are attracted to their host by carbon dioxide, and its believed that IJs of entomopathogenic nematodes are similarly attracted to their insect hosts. However, carbon dioxide is produced by virtually all living organisms in the soil, and so the strength and direction of gradients in such an environment is
large. If, therefore, carbon dioxide is the nonspecific attractant what is the specific agent that attracts IJs to their host, and is it the same or a different factor that retains the *Steinernema* spp. and *Heterorhabditis* spp. at the insect surface prior to their entry into the insect? These nematodes are strongly attracted also to plant roots where they may out-compete plant parasitic nematodes (4, 18). However, evidence suggests that microfloral products associated with the insect or plant may modify the target attractants for the IJs (E. Hui, pers. comm.) (Fig. 4a and 4b).

Once inside the insect the bacterial symbiont has a key role to play in the successful development of the nematode. The mechanisms leading to symbiont dietary preference by the nematode and to the preferential attachment of the bacterial symbiont to its respective symbiotic IJ intestine are only now beginning to be understood (9, 10). However, the mutualistic activities of the IJ and bacterium that help overcome the insect’s defence system and, subsequently, overcome bacterial and fungal competitors in the insect cadaver have received significant research attention in recent years (1, 5). Although there is debate over their relative and precise roles in this dynamic period of their reproductive life the general picture appears to be that bacterial toxins, together with other factors from the nematodes overcome the insect’s defences and the insect dies within 24-36 hrs. In the 24-96 hrs post infection phase bacteria derived antibiotics help overcome competition from alien microflora (6, 23). During this log phase of bacterial growth and during the 10 days postinfection the environment within the cuticular sac of the cadaver is maintained optimum for the production of large numbers of IJs.

These secondary metabolites with antibacterial and antifungal properties have significant economic potential for development as acceptable alternatives for the control of bacterial and fungal pathogens of agro-forestry crops. A large number of such secondary metabolites have been identified from the bacterial symbionts, and some of these are potential lead chemicals for new compounds with fungicidal action (7, 20) (Table 2). A mixture of some metabolites from *X. bovienii* were shown to have low phytotoxicity and to control potato blight, *Phytophthora infestans*, on potted potato plants (21) (Fig. 5).

Our understanding of this mutualistic, symbiotic relationship is leading not only to the

<table>
<thead>
<tr>
<th>Strains of <em>X. nematophilus</em></th>
<th>BC1</th>
<th>D1</th>
<th>ATC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (Days)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td><em>116.83</em> Ac</td>
<td>54.84 Bb</td>
<td>11.25 Cc</td>
</tr>
<tr>
<td>2</td>
<td>605.34 Aa</td>
<td>200.67 Ba</td>
<td>148.04 Ba</td>
</tr>
<tr>
<td>3</td>
<td>478.48 Aab</td>
<td>39.93 Cb</td>
<td>88.72 Bab</td>
</tr>
<tr>
<td>4</td>
<td>369.28 Ab</td>
<td>36.14 Bb</td>
<td>36.69 Bb</td>
</tr>
<tr>
<td>5</td>
<td>564.20 Aab</td>
<td>108.00 Bab</td>
<td>70.00 Bab</td>
</tr>
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* means in the same column with the same small letter or means in the same row with the same capital letter are not significantly different (n=4, P = 0.05) (after Li et al., 1997).
improved biological control of some insects pests but revealing the potential of this nematode-bacterium association in the integrated management of an array of agroforestry pests and pathogens. Successful biological control is the result of an intricate array of interactions that are strongly influenced by the local micro-environment. The soil habitat is a particularly complex environment with an enormous range of interacting rhizosphere communities that influence species diversity and population dynamics. The successful application of entomopathogenic nematodes and the products of their bacterial symbionts into an integrated pest management strategy will be an art as well as a science, and current progress and application of the new technologies is showing that it is possible.

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