Chapter - 8

General discussion
8. **General discussion**

The impetus for research in EPN and their symbionts has come about because of their biological control potential, so much of the focus in EPN research has been on applied aspects relating to pest control (Gaugler and Kaya, 1990; Bedding *et al.*, 1993). However EPN and their symbionts are increasingly being viewed as an exciting subject for basic research in ecology, biodiversity, evolution, biochemistry and molecular genetics. The bacterial symbionts produce novel insecticidal toxins, antibiotics and exoenzymes, but many of these bacterial species and strains are still unexplored. The molecular interactions between EPN and their symbiotic bacteria which enable the nematodes to package and transmit the bacteria are still largely unknown.

As the nematode/bacteria infestation kills the insect (host) with in 24-48 hr of infection, they must be producing a number of bioactive molecules toxic to insects. These invaders do not allow any type of competition from competing organisms found in the surrounding environment or even inside the gut of the dead insect. Always the nematode and bacteria were found to be as diaxenic cultured in the insect cadaver. Even when some other bacterial contaminations were found in the beginning, they were also eliminated very fast. No wonder the dead insect cadaver remain without putrifaction long enough for the bacteria and nematode to utilize the entire contents. A profile of bioactive molecules of the cell free culture filtrate/haemolymph of dead *G. mellonella* larvae indicated diverse compounds and they are able to suppress many organisms. As broad spectrum activity has been recorded (nematicidal, insecticidal, antibacterial, antifungal) these molecules may be even effective against many pathogens of humans and could be utilized for preparation of pharmaceutical drugs. As no work is done in these classes of bacteria, novel compounds are likely to be recorded. These are likely to be quite novel antimicrobial, anticancer and nematicidal molecules. These bacteria are found only in the specific nematodes and its insect host and do not exist elsewhere. Hence chances for having or developing resistance against most of the pathogens and pests are remote.
From this study it can be concluded that the nematodes are associated with specific bacterium. The nematode/bacterium combination can kill the insect larvae of *G. mellonella* with in 48 hr of infestation. The cadaver of the insects killed by the nematodes were found not to purify and always occupied by the specific bacterium and the nematode only. No other microorganisms were found inside the cadaver. Microbes present inside the insect gut as well as from the surrounding environment could not invade the cadaver indicating that wide spectrum antimicrobials are produced. These molecules produced by them are able to inhibit all microorganisms and kill the insect host while being non toxic to the nematode and also the bacterium.

There are no reports of the isolation of *Xenorhabdus* and *Photorhabdus* from soil samples, so it seems likely that bacteria may not be released from the nematodes or insects into the soil in significant numbers and/or that the bacteria may not survive for significant lengths of time in the free state. No systematic studies, have, however, been carried out to investigate either the occurrence of free bacteria in the soil or the survival capacity of *Xenorhabdus* and *Photorhabdus* in the soil (Burnell and Dowds, 1996).

In order to identify the bacteria symbiotically associated with new EPN isolate, genetic techniques along with phenotypic characterization of bacteria. Cyclic dipeptide (Diketopiperazine derivative) was isolated, purified and their structure elucidated from organic fractions of one of the bacteria. Two sub fractions eluted with dichloromethane/hexane and methanol/ethyl acetate found to be effective against pathogenic bacteria (*Bacillus subtilis* MTCC 2756, *Staphylococcus aureus* MTCC 902), fungi (*Aspergillus flavus* MTCC277, *Fusarium oxysporum* MTCC183), insect (*Galleria mellonella*) and plant parasitic nematode (*Meloidogyne incognita*).

**Future perspectives**

What pathways are critical to the relationship with these bacteria and not other bacteria? What nematode genes are critical to these interactions and how does the bacterium regulate mechanisms of killing to avoid killing the nematode partner? It is feasible to test the effect of every gene of the bacteria on the survival, reproduction and overall fitness of the nematode as an EPN. Further studies are also required to identify the bacterial genes responsible for the production of cyclic dipeptide. In order
to harness the potential of bioactive molecules for mitigating biotic stress in agriculturally important crop plants, the genes responsible for producing these compounds need to be identified so that it can be exploited in the crop improvement programme in future. Therefore the bacterium needs to be analysed at genome level. Such a genome wide identification of genes governing the biosynthesis of cyclic dipeptides and stilbenes assume significance in the technology empowered 21st century. The methodologies of classical genetics and genetic engineering can be used for the genetic improvement of EPN and their symbiotic bacteria.