STUDIES ON SOME SYMBIOTIC BACTERIA OF SOME ENTOMOPATHOGENIC NEMATODES

By

GEHAN MOHAMED SAYED AHMED
B. Sc. Agric. Sci., (Biotechnology), Fac. Agric., Cairo Univ., 2004

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APPROVAL SHEET

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Title of Thesis: Studies on Some Symbiotic Bacteria of Some Entomopathogenic Nematodes
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ABSTRACT

In this work, *Xenorhabdus* spp and *Photorhabdus* spp. were isolated from a local nematode strain *Steinernema* sp. , *Heterorahbitids* sp , a foreign *Heterorahbitids* sp, a foreign well-identified strain, *i.e*., *Steinernema riborvae* and *Heterorahbitids indica* kindly supplied by Florida State University, USA. The identity of the isolated bacteria was authenticated referring to their cultural, morphological and biochemical traits as well as to their entomopathogenicity against *Galleria mellonella, Agrotis ipsilon, Spodoptera littoralis* and *Rhynchophorus ferrugineus.* The 16S rRNA technique was adopted for conclusive identification of two *Xenorhabdus* spp strains and three *Photorhabdus* spp. The antagonistic properties of the isolated *Xenorhabdus* spp and *Photorhabdus* spp. isolates were studied in submerged batch fermentations . Five periods were tested for selection of the ideal time of *Photorhabdus* sp. (xH1, xHi and xH2), *Xenorhabdus* sp. (xSr and xS1) to give their maximum antibiotic activity using four different fermentation media using the Disc Diffusion Technique. Five *Xenorhabdus* spp and *Photorhabdus* spp. strains showed various antimicrobial activities against wide spectra of Gram +ve, Gram –ve bacteria as well as yeasts. Oral administration of cell-free culture supernatants of *Xenorhabdus* spp. isolates exhibited high levels of toxicity against *Agrotis ipsilon* and *Spodoptera littoralis*. All toxins achieved high mortality within 72h after treatment. Histopathological effect of *Xenorhabdus* spp and *Photorhabdus* spp strains on the midgut of *Spodoptera littoralis* was examined.

Key words :*Xenorhabdus* spp., *photorhabdus spp.,* oral toxicity, antimicrobial activities.

DEDICATION

I dedicate this work to whom my heartfelt thanks; to my father, my mother, Dr. Olfat , my dear mai , my sisters, my brothers and my friends for their patience, help and for all the support they lovely offered along the period of my post graduation.

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INTRODUCTION

The indiscriminate use of agrochemicals in intensive agriculture causes a global serious environmental problem. Many pesticides survive in plants for long periods, enter the food chain, and so can be detected at high levels in many feed and food crops, meat, and dairy products. Such environmental pollution hazard with profound effects on human health is severe in developing rather than developed countries. Many bacterial and fungal bioinsecticides are commercially available for use in biological control of wide spectra of plant pests; however, exploring new biological control agents is badly needed. Among bacterial bioagents are the Gram-ve bacteria *Photorhabdus* sp. and *Xenorhabdus* sp.; the microsymbionts of the entomopathogenic soil nematodes from the families *Heterorhabditis* and *Steinernema* (Forst *et al.,* 1997). These bacteria produce insecticidal factors critical for their pathogenic activities against insects (ffrench-Constant *et al.,* 2007). The nematodes enter the openings of the insect body, such as the mouth, spiracle, or anus where nematodes regurgitate bacteria, which are housed in a vesicle of their intestine, directly into the hemocoel (Ciche and Ensign, 2003). The bacteria produce a range of proteins and metabolites which kill the insect host (Bowen *et al.,* 1998). Both nematodes and bacteria replicate in the insect cadaver (Ffrench-Constant *et al.,* 2003). *Photorhabdus* and *Xenorhabdus* bacteria can be isolated from the infective juvenile nematodes that carry them or from the infected insect cadaver. They can be *in vitro* cultured as free living without hosts under laboratory conditions (Forst *et al.,* 1997). The

bacteria secrete entomopathogenic factors directly into the growth medium. Interestingly, these bacteria or their toxic factors are insecticidal when they are ingested through the insect mouth or when injected into the hemolymph (Ffrench- Constant *et al.*, 2003). Oral toxicity of *Photorhabdus luminescens* and *Xenorhabdus luminescens* was reported to be lethal to *Manduca sexta* larvae (Blackburn *et al.*, 1998). Cells and their secreted proteins of *Xenorhabdus nematophilus* were described as orally toxic to neonatal larvae of *Helicoverpa armigera* (Khandelwal and Banerjee-Bhatnagar, 2003). A possible exploitation of *Xenorhabdus* and *Photorhabdus* as microbial biopesticides in agriculture was adopted by Ffrench-Constant *et al.* (2007). However, there is no much data on the insecticidal capabilities of native entomopathogenic nematodes and their microsymbionts isolated from the Egyptian soils and it is desirable to study the native potential biocidel characteristics of the entomopathogenic nematodes and their microsymbionts.

In this work, it was planned to isolate *Xenorhabdus* and *Photorhabdus* from their nematode symbionts and study their cultural, morphological, biochemical and molecular characteristics.Using two insect genotypes, an experiment was conducted for studying the oral toxicity of *Xenorhabdus* and *Photorhabdus* cultures as well as the activity of their cell-free filtrates on nematode and insect host. Furthermore, the antagonistic activities of these bacteria were *in vitro* examined against some microorgansms.

REVIEW OF LITERATURE

1. Entomopathogenic nematodes and their microsymbionts

Burnell and Stock (2002) reported that the entomopathogenic nematodes (EPN) *Heterorhabditis* and *Steinernema* together with their symbiont bacteria *Photorhabdus* and *Xenorhabdus*, respectively, are obligate and lethal parasites of insects. EPN can provide effective biological control of some important lepidopteran, dipteran and coleopteran pests of commercial crops and they are amenable to large-scale culture in liquid fermentors.They are unique among rhabditidsin having a symbiotic relationship with an enteric bacterium species. The bacterial symbiont is required to kill the insect host and to digest the host tissues, thereby providing suitable nutrient conditions for nematode growth and development.

Mathieu *et al.* (2006) showed that *Xenorhabdus* symbionts modified the competition between their *Steinernema* associates. This suggests that *Xenorhabdus* not only provides *Steinernema* with access to food sources but also furnishes new abilities to deal with biotic parameters such as competitors.

Stock *et al.* (2008) decoumented that Entomopathogenic nematodes *Steinernema* and *Heterorhabditis* spp.(Nematoda: *Steinernematidae*, *Heterorhabditidae*) and their bacterial symbiont *Xenorhabdus* and *Photorhabdus* spp.Gram-negative*Enterobacteriaceae* represent an emerging model of terrestrial animal-microbe symbiotic relationships. *Xenorhabdus* and *Photorhabdus* spp. are harbored as