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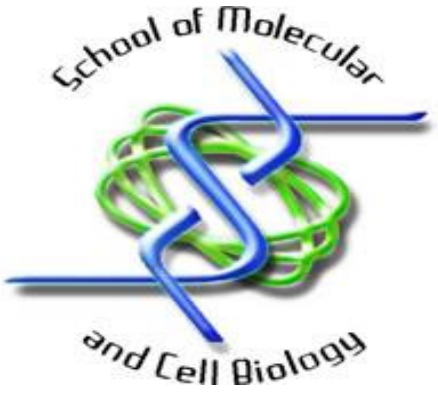
Characterization, host bioassay, and in vitro culture of indigenous entompathogenic nematodes and their bacterial symbionts

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UNIVERSITY OF THE WITWATERSRAND



Characterization, Host Bioassay, and *In vitro* Culture of Indigenous Entompathogenic Nematodes and their Bacterial Symbionts.

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0413449k

A dissertation submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Science.

October 2008

DECLARATION

I declare that this research report is my own unaided work. It is being submitted for the degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted for any degree or examination at any other university.

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Lubanza Ngoma Date

ABSTRACT

The prevailing use of chemical pesticides has generated several problems including insecticide resistance, outbreak of secondary pests, safety risks for humans and domestic animals, contamination of ground water and decrease in biodiversity among other environmental concerns (Webster, 1982). These problems and the non-sustainability of control programs based mainly on conventional insecticides have stimulated increased interest in the development and implementation of cost-effective, environmentally safe alternatives to chemical pesticides for insect pest control. One of the most promising strategies to help minimize dependence on chemical pesticides has been the recent application of entomopathogenic nematodes (EPNs) as biocontrol agents. EPNs in the families Steinernematidae and Heterorhabdidae have been shown to have considerable potential as biological control agents. As a natural process, biological control has the potential to play an important role in the suppression of field crop pests in agriculture. EPNs as biocontrol agents have the following advantages: high virulence, safety to non target organisms, ability to search for hosts, high efficacy in favourable habitats, high reproductive potential, ease of mass production, ease of application (Ferron & Deguine, 1996).

To isolate the EPNs in South African soil, 200 soil samples were randomly collected from 5 locations in the agricultural research council (ARC) Pretoria, Gauteng province in April 2006; and 5 locations in Brits, North West province in March, 2006. At the different collection sites, soil samples were obtained from soils associated with various types of vegetation. The nematodes were collected from sandy soil by the insect-baiting technique and maintained successfully *in vivo* for 12 months on *Galleria mellonella* (*G. mellonella*), 4 months on *Tenebrio molitor* (*T. molitor*); 2 months Pupae and in *vitro* (lipid agar) for 2 weeks in the laboratory. Out of a total of 200 soil samples that were baited, 2 were found to be positive for EPNs.

In addition to completing Koch's postulates, the colour of cadavers infected by the putative EPNs were also used as a diagnostic characteristic for categorizing the nematode isolates. Characterization and identification of the EPN isolates were based on morphological characters, as well as on a molecular marker (18S rDNA).

On the basis of the morphological and molecular data that was obtained both of the EPNs isolates were placed in the family Heterorhabdidae: *Heterorhabditis bacteriophora* (*H. bacteriophora*) and *Heterorhabditis zealandica* (*H. zealandica*). Also from the phylogenetic trees generated from the 18S rDNA sequence, the indigenous putative *H. bacteriophora* was shown to be closely related to *H. bacteriophora* (accession number EF690469) and indigenous putative *H. zealandica* to *H. zealandica (*accession number AY321481). The two EPNs were found associated with Gram negative rod-shaped bacteria. The bacterial symbionts of the two isolates were isolated and a region of the 16S rDNA gene was sequenced. National Center for Biotechnology Information (NCBI-BLAST) results of the 16S rDNA sequence obtained showed the endosybiotic bacteria to be *Photorhabdus luminescens laumondii* (*P. laumondii*) (*H. bacteriophora*) and *Photorhabdus* sp (*H. zealandica*). Results of the tree showed that isolates from *H. bacteriophora* appeared to be closely related to *P. luminescens* subsp *laumondii* strain TT01 Ay 278646. The isolates from *H. zealandica* appeared to be most closely related to *Photorhabdus* sp (Accession number: Q 614 Ay 216500).

Bioassays were used to determine the infectivity of the two EPNs. In this experiment different infective juvenile (IJs) concentrations (5, 10, 25, 50, 100,200 400 and 500) of the two EPNs were applied per *G. mellonella*; *T. molitor* larva and pupae. The bioassay was carried out in two parts. In the first part, mortality data was collected for *H. bacteriophora* and *H. zealandica*. The results showed that the degree of susceptibility of *G. mellonella*, *T. molitor* larvae and pupae to each nematode species was different. When 24 h post-exposure mortality data for larvae exposed to the IJs of *H. bacteriophora* and *H. zealandica* were analyzed, ANOVA showed no differences

in mortality between insects exposed to different *H. bacteriophora* IJ doses (Fig: 8.1 ABC). However, there were significant differences in mortality between insects exposed to different IJ doses of *H. zealandica* such as 5 and 500 IJs/insect (Fig: 8.2 ABC) Therefore, no differences were noted when mortality data was compared between IJ doses at both 72 h and 96 h following IJ application to the insects. The highest susceptibility was observed with *G. mellonella* followed by *T. molitor* pupae and then *T. molitor* larvae. According to Caroli *et al*., (1996), the total mortality of insect such as *G. mellonella* and other lepidopterans, was reached within 24-72 h of exposure to nematodes at concentrations such as those tested here. In this study similar results were observed with high concentration of nematodes (100, 200 and 500). In the second part of the dose response bioassay, the number of progeny IJs emerging from EPN-infected cadavers was determined for all two EPNs.

The results indicate that IJ progeny production differed among the three insect hosts used, the IJ doses they were exposed to, as well as the EPN species (Figs 8.3 & 8.4). The highest number of emerged IJs of *H. zealandica* was produced by *G. mellonella* (mean ± SEM: 220500 ± 133933 IJs), followed by *T. molitor* larvae (mean ± SEM: 152133 ± 45466 IJs) and the lowest then *T. molitor* pupae (mean ± SEM: 103366 ± 56933 IJs).

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DEDICATION

This thesis is dedicated to my daughter Divine Lubuya and my wife Susan Tshiame. They have been my inspiration and motivation throughout this work. I love you both.

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LIST OF ABBREVIATIONS

df	Degrees of freedom
DNA	Deoxyribonucleic acid
EDTA	Ethylene Diamine Tetra-Acetate
EPN(s)	Entomopathogenic Nematode(s)
IJ(s)	Infective Juvenile(s)
PCR	Polymerase Chain Reaction
IMP	integrated pest management
rRNA	Ribosomal Ribonucleic acid
rpm	Revolutions per minute
TBE	Tris-Borate-EDTA
TE	Tris-EDTA
V	Volts
WHO	World Health Organization
RH	Relative Humidity
UV	Ultraviolet Radiation
NBTA	bromothymol blue and triphenyltetrazolium chloride medium
ARC	Agricultural Research Council
ITS	internal transcribed spacers
TAF	Triethanolamine-formalin
SEM	Scanning electron microscopy
Tm	Melting temperature