COLLECTION OF ENTOMOPATHOGENIC NEMATODES FOR THE BIOLOGICAL CONTROL OF INSECT PESTS

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A B S T R A C T

The demand for biological control agents is increasing all over the world. In many cases, however, research can not keep up with the expectations of growers. To elaborate a new biological control technique, many species and strains of potential control organisms have to be evaluated. In Hungary, the lack of detailed and reliable biogeographic data and the lack of a usable strain collection seriously restrict research on biological control techniques. The goals of our project are to survey the entomopathological nematodes occurring in Hungary and to establish and maintain a collection of entomopathological nematodes and bacteria which can serve as the basis for further research on the biological control of insect pests, especially grubs of *Melolontha melolontha* (Coleoptera: Scarabeidae). Entomopathological nematodes and bacteria were collected from different parts of Hungary and identified using molecular methods. Pathogenicity to various insect hosts was then determined. The nematodes are maintained in a strain collection and stored in liquid nitrogen. These collections of entomopathological nematodes and bacteria are freely available to other research institutes.

Key words: biological control agents, entomopathogenic nematodes, strain collection

INTRODUCTION

For over ten years, entomopathogenic nematodes (EPNs) belonging to the families Steinernematidae and Heterorhabditidae (Rhabditida) have been used as effective biological control agents against a wide spectrum of insect pests. Steinernematids are symbiotically associated with entomopathogenic bacteria (EPBs) from the genus *Xenorhabdus,* and heterorhabditid nematodes are symbiotically associated with EPBs from the genus *Photorhabdus.* The bacterial symbionts produce wide range of toxins, hydrolytic exoenzymes, and antibacterial compounds. These compounds not only kill and bioconvert

infected larvae, but also preserve the cadavers from being consumed by other soil organisms. There have been recent advances in the technology of mass producing and formulating nematodes (Grewal 2002, Ehlers 2001). These recent advances, together with the need to reduce pesticide use, have resulted in a surge of scientific and commercial interest in EPNs and their symbiotic bacteria.

About thirty species of Steinernematidae and nine species of Heterorhabditidae have been described so far (Adams and Nguyen, 2002). More than ten *Steinernema* species and three *Heterorhabditis* species are found in Central Europe (Hominick, 2002; Mracek et al., 2005). In Hungary, only two surveys steinernematid and heterorhabditid nematodes have been carried out so far. Only three species have been identified (*S. feltiae*, *H. bacteriophora* and *H. megidis*) (Mracek and Jenser, 1988; Griffin et al., 1999).

Many species and strains of potential control organisms have to be evaluated to elaborate a new biological control technique. In Hungary, the lack of detailed and reliable biogeographic data and the lack of a usable strain collection seriously restrict research on biological control techniques.

The goals of our project are to survey the EPN fauna in Hungary and to establish and maintain a collection of EPNs and EPBs which can serve as the basis for further research on the biological control of insect pests, especially grubs of *Melolontha melolontha* (Coleoptera: Scarabeidae). The first results of this project are presented in this paper.

MATERIAL AND METHODS

Isolation of nematodes

Soil samples were collected from different parts of Hungary. 89 samples were collected in 2003, and 200 samples were collected in 2005. All sampling sites were habitats typically preferred by *Melolontha melolontha,* including the peripheries of oak and other deciduous forests, new plantations, and fruit orchards. Five random samples were collected at each site and transported to the laboratory in plastic bags.

In 2003, three late instar larvae of *Galleria mellonella* (Lepidoptera: Pyralidae) in a porous Eppendorf vial were put into each sample bag. In 2005, three larvae of *G. mellonella* and two larvae of *Tenebrio molitor* (Coleoptera: Tenebrionidae) were put into each sample. After seven days, larval mortality was recorded. Dead larvae were cultured in a water trap to obtain infective juvenile nematodes, which were stored suspended in water in a refrigerator at 5 to 7°C.

Methods of identification

RFLP PCR was used to identify the nematodes down to the species level on the basis of the ITS region of the genome (Hominick et al., 1997). The

number and size of DNA fragments produced by digesting *Steinernema* DNA with six restriction endonucleases are known (Hominick et al., 1997). Reference data have also been reported for *Heterorhabditis* (Adams et al., 1998).

Methods of long-term storage

Two strain banks are maintained: a master strain bank and a working strain bank. In the master strain bank, nematodes are maintained as cryopreserved specimens using methods described elsewhere (Curran et al., 1992; Bai et al., 2004). In the working strain bank, which is used for ongoing studies on taxonomy and pathology, nematodes are maintained suspended in water in the refrigerator. The collection conforms to general Hungarian and European Union standards (EN 1619:1996). In order to avoid accidental loss, all strains are stored in duplicate in separate storage units.

All strains are regularly tested for viability and infectivity. For each strain, records are kept on collection site, collection date, morphology, taxonomy and molecular biology in the form of RFLP patterns or sequences of relevant DNA regions. Other data include host range, if known, and virulence towards selected insect larvae such as *Galleria mellonella* and *Tenebrio molitor*.

RESULTS

EPNs were isolated from 99 of 289 sampling sites (Tab. 1). In 2003, one steinernematid and three heterorhabditid species were found (Tab. 2). The nematodes isolated in 2005 are still being identified. The most common species was *S. feltiae*, which made up 79.5% of the nematodes isolated. Among the nematodes isolated was *H. downesi,* which had never been reported to occur in Hungary.

Table 1. Number of nematode isolates in the strain collection isolated in 2003 and 2005

	2003	2005
Number of sampling sites	89	200
Number of positive sites*	31	68
Number of isolates in the working strain bank	60	237 (41)**
Number of isolates in the master strain bank	39	– ***

*number of sampling sites, where nematodes originated from
**number in parentheses indicates the nematode isolates baited by *Tenebrio molitor* larvae
***ongoing work

Table 2. Species in the strain collection isolated in 2003

Species	Number of strains
Heterorhabditis bacteriophora	1
Heterorhabditis downesi	2
Heterorhabditis megidis	5
Steinernema feltiae	31

DISCUSSION

Entomopathogenic nematodes infect over 200 insect hosts (Shapiro-Ilan et al., 2002). Nevertheless, nematodes have been successfully marketed only for a small fraction of these insects and only in restricted parts of the world. Active biocontrol programs have been implemented mainly in western Europe and the USA (Kaya et al., 2005). The main reasons why nematodes are not more widely used to control more insect species are the lack of detailed and reliable biogeographic data and the lack of usable strain collections for research. The present project is the first systematic survey of the EPN fauna in Hungary. The project was motivated by the need to biologically control some key insect pests, including *Melolontha melolontha*. Preliminary results of this ongoing project are presented in another paper in this volume (Lakatos and Tóth, 2005).

Only three EPN species had been previously reported to occur in Hungary (Mracek and Jenser, 1988; Griffin et al., 1999). This is low compared to other central European countries such as the Czech Republic, where eleven species have been identified (Mracek et al., 2005). In 2003, one new *Heterorhabditis* species was found, which means that all of the palearctic *Heterorhabditis* species can be found in Hungary (Hominick, 2002). When the field samples collected in 2005 have been identified, our knowledge about the EPN fauna in Hungary will be complete.

The symbiotic bacteria of EPNs, their entomotoxins and the antibiotics they produce are also extremely interesting (Webster et al., 2002). Our strain collection makes it possible to carry out pure and applied research on these bacteria. Work is currently underway to isolate entomopathogenic bacteria from nematodes and assemble them in a strain collection.

Our institute has excellent conditions for maintaining microbiological strain collections. Storage in liquid nitrogen is the only way to preserve the original properties of newly isolated strains, including storage stability, reproduction potential, virulence, and stress tolerance. It also prevents inadvertent selection or genetic deterioration during laboratory maintenance (Wang and Grewal, 2002).

Our collections of entomopathological nematodes and bacteria are freely available to other research institutes. Please contact the author for further details.

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KOLEKCJA NICIENI ENTOMOPATOGENICZNYCH DO BIOLOGICZNEGO ZWALCZANIA SZKODNIKÓW

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STRESZCZENIE

Na całym świecie obserwuje się wzrost zapotrzebowania na czynniki biologiczne stosowane do ochrony roślin. Jednak w wielu przypadkach nauka nie jest w stanie sprostać oczekiwaniom producentów. Opracowanie nowej techniki biologicznego zwalczania wymaga oceny wiele gatunków i ras potencjalnych organizmów, które mogą do tego być wykorzystane.

Na Węgrzech, brak szczegółowych i pewnych danych biogeograficznych, jak również brak kolekcji użytecznych ras, ogranicza badania nad technikami biologicznego zwalczania. Celem naszego projektu jest sprawdzenie entomopatogenicznych nicieni występujących na Węgrzech oraz założenie i utrzymanie kolekcji nicieni entomopatogenicznych i bakterii, które będą stanowiły podstawę do dalszych badań nad biologicznym zwalczaniem szkodników, szczególnie pędraków chrabąszcza majowego *Melolontha melolontha* (Coleoptera: Scarabeidae). Entomopathogeniczne nicienie i bakterie były zebrane z różnych części Węgier i zidentyfikowane metodą molekularną. Określano także patogeniczność nicieni w stosunku do różnych owadów gospodarzy. Poszczególne rasy tych nicieni są przechowywane w ciekłym azocie. Kolekcja entomopatogenicznych nicieni i bakterii jest udostępniana innym placówkom badawczym.

Słowa kluczowe: czynniki biologicznego zwalczania, nicienie entomopatogeniczne i bakterie, kolekcje ras