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Entomopathogenic nematode *Steinernema carpocapsae* (Weiser) (Rhabditida: Steinernematidae), a new member of Slovenian fauna

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ABSTRACT

In April 2008, 120 soil samples from 24 locations were collected in Gorenjska, Notranjska and Primorska regions as well as in Ljubljansko barje. The presence of entomopathogenic nematodes was confirmed in 9 samples from 6 locations. Only the sample C101, which was taken in the village Svino in the area of Breginjski kot (western part of Slovenia, the vicinity of Italian border), was sent to genetic analysis. Molecular biological analysis was proved the identity of the sample with the species *Steinernema carpocapsae* (Weiser). This was the first record of *Steinernema carpocapsae* in Slovenia. In preceding researches on the fauna of entomopathogenic nematodes in Slovenia, which started in 2007, we already established the occurrence of *Steinernema affine* (Bovien) and *Steinernema feltiae* (Filipjev).

Key words: entomopathogenic nematodes, Slovenia, *Steinernema carpocapsae*, biological control

IZVLEČEK

ENTOMOPATOGENA OGORČICA *Steinernema carpocapsae* (Weiser) (Rhabditida: Steinernematidae), NOVI PREDSTAVNIK SLOVENSKE FAVNE

V aprilu 2008 smo na območju Gorenjske, Notranjske, Primorske in Ljubljanskega barja na 24 lokacijah nabrali 120 talnih vzorcev. Zastopanost entomopatogenih ogorčic smo ugotovili v 9

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vzorcih s 6 lokacij. V nadaljnjo genetsko analizo smo poslali le vzorec C101. Ta je bil odvzet v vasi Svino na območju Breginjskega kota (skrajni zahod Slovenije na meji z Italijo). Z molekulsko analizo smo identificirali vrsto *Steinernema carpocapsae* (Weiser). Gre za prvo odkritje omenjene vrste entomopatogene ogorčice pri nas. V predhodnjih raziskavah preučevanja faune entomopatogenih ogorčic v Sloveniji, ki potekajo od leta 2007, smo ugotovili zastopanost vrst *Steinernema affine* (Bovien) in *Steinernema feltiae* (Filipjev).

Ključne besede: entomopatogene ogorčice, Slovenija, *Steinernema carpocapsae*, biotično varstvo

1 INTRODUCTION

It is well known that entomopathogenic nematodes, which are classified into Steinernematidae and Heterorhabditidae families, have great potential as biological control agents in plant protection (Klein, 1990). Their activity against different pest insects is already well studied (Kaya in Gaugler, 1993; Ebssa, 2005). Entomopathogenic nematodes are soil organisms, which live with bacteria in symbiotic-mutualistic relationship. For the first time their importance in biological control was discovered in the United States of America in the thirties of past century (Laznik in Trdan, 2008).

At the time of the first record of entomopathogenic nematodes, a hypothesis was raised that these agents by themselves cause death of attacked insects (Gaugler in Kaya, 1990). In 1937, Bovien first mentioned a possibility of symbiotic bacteria to live in mutualistic relationship with entomopathogenic nematodes. His hypothesis was confirmed in 1955 by Dutky and Weiser (Weiser, 1955). In 1982, Boemare gave a proof for production of toxic substances by the nematodes from genus *Steinernema*. These substances have negative influence on the immune system of infected insect and could also cause death of the host without the presence of symbiotic bacteria. Until now no evidence was given that nematodes from genus *Heterorhabditis* are capable for their own production of toxins, which would be able to influence a poor viability of invaded insects (Klein, 1990).

It is discussed upon symbiotic-mutualistic relationship because nematodes provide shelter and protection for bacteria in an exchange for killing the attacked insects. Latter, bacteria also produce antibiotics, which prevent the development of intra- and interspecific competitors, which would also feed on cadavers. Bacteria transform the content of the host into feed, suitable for nematodes and also bacteria themselves are feed for nematodes (Kaya in Koppenhöfer, 1999). Nematodes from the family Steinernematidae live in symbiosis with bacteria from genus *Xenorhabdus*, meanwhile it is well know for the ones from the family Heterorhabditidae that they have the same association with bacteria from genus *Photorhabdus* (Forst *et al*., 1997).

In Slovenia, momentarily only entomopathogenic nematode *Steinernema feltiae* has a status of indigenous species (MAFF, 2008); therefore only this species can be applied in the field. With the researches, which results we also present in this paper, we want to enlist as more species of entomopathogenic nematodes as it is possible,

while in foreign countries they worth as alternatives to insecticides in controling pest insects. The strain C101, which we present in a current paper, we plan to use in extensive experiments in the future; first in the laboratory and afterward, when its status will be administratively entrenched, also in the field.

2 MATERIALS AND METHODS

In April 2008, we examined 120 soil samples from 24 different locations on the occurrence of EPNs in Slovenia. The soil samples, four from each region, were taken in Gorenjska, Notranjska and Primorska region as well as in Ljubljansko barje. We used »Galleria bait method«, which is the most frequently used method of EPNs detection from soil. After the death of greater wax moth (*Galleria mellonella* [Linnaeus]) larvae, we dried cadavers for 12 days and put them in so called »White trap« (Bedding and Akhurst, 1975) to separate the nematodes from death larvae. The suspension, which was acquired in this way, was used for artificial infection of the larvae of greater wax moth. Following procedure contained the use of centrifuge and 5 % concentration of sodium hypochlorate. The aim of this process was to acquire infective juveniles from the suspension. We confirmed the presence of nematodes in 9 soil samples of 6 locations. Only 1 positive sample, C101 (taken on the arable field near village Svino in Breginjski kot (NW Slovenia, 46°14′N, 13°33′E, 285 m alt.) was identified to this time.

3 RESULTS

To confirm the identification of isolated nematodes from larvae of wax moth, a selected sample was analysed by molecular biological approach. Genomic DNA was extracted from individual nematodes and PCR was performed to multiply ITS region using primers TW81 and AB28 after Hominick et al. (1997). PCR product were reisolated from 1 % TAE-buffered agarose gel using QIAquick Gel Extraction Kit (Qiagen, USA). Reisolated sample was sequenced in the laboratory of Biological Research Centre in Szeged, Hungary. The sequence was submitted into GenBank public database (Accession Number: EU914854). Sample DNA sequence was compared to sequences of species *Steinernema* using BLAST search in National Centre for Biotechnology Information (NCBI) web site (www.ncbi.nlm.nih.gov). The sequences producing significant alignments and at least 99 % identity were derived from *Steinernema carpocapsae*: GenBank Accession No. AY171282 and EU122951 (Spiridonov *et al*., 2004) (Fig. 1).

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9833      1    GGAA-GA-CATTATTGAGCTAATATT-TTCC-TTTTCT-ATCAAGT-
TTTCGCTGCTCGT  54
AY171282  1                 ............-....-....-.A.......-
........T....  43
EU122951  103  ....G..T................-....-....-.A.......-
........T....  158
AY171280  1                 ..........-..C....A....-A-
....G..C...-....T....  43

9833      55   TTCTAAGCTTTAACTTGACCTC-
TAACGGCTTTGAAAGGTTTCTACAGATGTTTGGAGCA  113
AY171282  44   ................T...-
.....................................  102
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EU122951  159  ................-G...-
.................................  216
AY171280  44   ........A..G...C..--..-.---
T........T........T...C.........  97

9833      114  GTTG-TAT-GAGCGTGACTGTGCTGATGAA-
CATTGTACATTGTTATCTAAGC-GTTTCG  169
AY171282  103  ....-...-....................-
.....................-......  158
EU122951  217  ....-...-....................-
.....................-......  272
AY171280  98   ..CA-.-.CA...........G....-..G.G..T...T...C.-
.-.---..A.-....  149

9833      170  AT-
GTTTCTAGAATGCTTAGTGATGAGAATTAAAGAGGTCTGCTGACTCGCCATTCTTTG  228
AY171282  159  ..-
.....................................................  217
EU122951  273  ..-
.....................................................  331
AY171280  150  -
.C......T..........C..............A..................-....
207

9833      229
ATTGCTAACAAAAACGTTTTGTTTCGATAATTGTGTCACTCGTTGATGCATTTTTTAAA-
287
AY171282  218
.....................................................T-
276
EU122951  332  ..............-......G..-.......-.....-
...........-.....-..-  384
AY171280  208
.....................T...TT.........T......A....A..C..T-
266

9833      288  NATC-
AAGTCTTATCGGTGGATCACTCGGTTCGTAGGTCGATGAAAAACGGGGCAAAAAC  346
AY171282  277  T...-
.....................................................  335
EU122951  385  TT..-......A.............................-
..........-.....  441
AY171280  267  T...-
.....................................................  325

9833      347
CGTTATTTGGCGTGAATTGCAGACATATTGAGCGCTAAAATTTTGAACGCAAATGGCACT
406
AY171282  336
.....................................................
395
EU122951  442  .....................-.............-
.................  499
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AY171280  326
..........................................................
385

9833      407
AACAGGTTTTTATCTGTTAGTATGTTCAATTGAGGGTCTTTTGACTAGAATCTGGCAATC
466
AY171282  396
..........................................................
455
EU122951  500  ......-
.....................................................  558
AY171280  386  ......G..--
.............................................  443

9833      467  CGCTGTGATTGCTTTTTCGGTAA-GCTACTTTGCT-T-T--T-----
--G---T-G--AA  508
AY171282  456  G.....................-..........-.-.--.-----
--.---.-.--..  497
EU122951  559  G.....................-..........-.-.--.-----
--.---.-.--..  600
AY171280  444  G............C.....A..-...........-.-AG.-----
--.---.A.TG..  490

9833      509
GTACCTTTTCNGTATGGCTATTTGATTGTCTAACGGATGTCTGGCTAGCTGCTTCTTTGC
568
AY171282  498
..........G.....................T.................T........
557
EU122951  601
..........G.....................T.................T........
660
AY171280  491
..........G.................A...T..........T......T........
550

9833      569  TAGACGTCTGCAATCATTCGGCATTGCGTAGTGTTTGATTAAT-
GGTTTAGCGCGTTTCT  627
AY171282  558  ................T.......................-
..............  616
EU122951  661  ................T.......................-
..............  719
AY171280  551
................T...T.............AC...A....-..........
609

9833      628
TGCTAACTGACTTTTACACAAGCAAGTGTAATACGTTTCTTAAAGTCAGCTCATTAATCA
687
AY171282  617
..........................................................
676
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EU122951  720
..........................................................
779
AY171280  610
..............G..............T........G.........-....T...
668

9833      688  ATGTGGTTTTCTGACTTGATTTGTC-
GGTCAATTGTGCTATGCTCTG-CTAATCTTTTCG  745
AY171282  677  ........................-
.....................-...........  734
EU122951  780  ........................-
.....................-...........  837
AY171280  669  ..T............C.C......-
...TT.C.............T.-..........  726

9833      746  AACT-AGACCTCAATT-GAGC  764
AY171282  735  ....-.......           745
EU122951  838  ....-...........T....  857
AY171280  727  ....-.........         739
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Figure 1: Multiple sequence alignment of the ITS rDNA region (including partial fragments of the 18S and 28S rRNA genes) of 4 *Steinernema* species. Code 9833 correspond to the Slovenian isolate of *Steinernema carpocapsae* (C101). Codes AY171282 and EU122951 are *Steinernema carpocapsae* strains from Russia and Iran, respectively. Code AY171280 correspond to *Steinernema tami* strain from Vietnam.

4 DISCUSSION

Genetic studies proved that the nematode species is *Steinernema carpocapsae* (Weiser, 1955) (Fig. 1). The ITS1-5.8S-ITS2 region, including the partial 18S and 28S rRNA genes (flanked by above primers) of Slovenian isolate C101 is 746 bp long. BLAST searches (Altschul *et al*., 1990) in GenBank showed that Slovenian isolate C101 (Fig. 2) has a high similarity (99 %) with those sequences available for *S. carpocapsae* populations (e.g. accession numbers AY171282 and EU122951). Sequence of other species from *carpocapsae* group, namely *S. tami* was obtained from GenBank searches that exhibited a lesser degree of similarity with the Slovenian isolate and other *S. carpocapsae* populations (e.g. accesion number AY171280) (Fig. 1). The present study constitutes the first report of *S. carpocapsae* in Slovenia. *S. carpocapsae* has a wide distribution in temperate regions, being one of the most common species found in Europe (overall in 15 countries), and in many other parts of the world (for a detailed EPN species distribution see Hominick, 2002).

We can place mentioned species into »*carpocapsae* group« of nematodes from genus *Steinernema* (Nguyen, 2006); for infective juveniles it is known that they are less than 600 µm long (Fig. 2). This nematode lives in symbiosis with bacterium *Xenorhabdus nematophila* (Akhurst, 1980). The nematode was first recorded in

1955. *Steinernema carpocapsae* is the most studied, available, and versatile of all entomopathogenic nematodes (Gaugler, 2002).

Figure 2: Entomopathogenic nematode *Steinernema carpocapsae* (strain C101).

Important attributes include ease of mass production and ability to formulate in a partially desiccated state that provides several months of room-temperature shelf-life (Gaugler et al., 2002). Particularly effective against lepidopterous larvae, including various webworms, cutworms, armyworms, girdlers, and wood-borers (Georgis *et al*., 1991). This species is a classic sit-and-wait or "ambush" forager, standing on its tail in an upright position near the soil surface and attaching to passing hosts (Campbell *et al*., 2003). Consequently, *S. carpocapsae* tends to be most effective when applied against highly mobile surface-adapted insects. Highly responsive to carbon dioxide once a host has been contacted, the spiracles are a key portal of host entry. It is most effective at temperatures ranging from 22 to 28 °C (Georgis *et al*., 1991).

In Europe, the occurrence of *S. carpocapsae* was up to now confirmed in Austria, Bulgaria, Czech Republic, France, Germany, Great Britain, Italy, Norway, Poland, Portugal, Slovakia, Spain, Sweden and Switzerland (Hominick, 2002). Among entomopathogenic nematodes only *Steinernema feltiae* has a status of indigenous species for the time being (Laznik et al., 2008; MAFF, 2008). While in Slovenia, the effectiveness of this species was already tested in the field experiment (Laznik, 2008, unpubl.), this was yet not the case for *S. carpocapsae*. When also the latter species will shift from exotic agents list, we will test its activity against the pest insects in the open too. The most intensive experiments will be done against these insect pests against which we do not have registered insecticides, their number is limited, and specially against the insects, which already developed the resistance to insecticides.

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