Scientific Report

Short Term Scientific Mission, COST 850

title: Screening for intron polymorphisms in nematode *Steinernema kraussei*

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Introduction

Throughout the Budejovicka basin, where Ceske Budejovice city is situated, the entomopathogenic nematode *Steinernema kraussei* often inhabits edges of coniferous forests formed by oak trees. These sites are separated by large field or wet meadow areas, which probably form borders between particular *S. kraussei* populations.

The future study's objective is to evaluate the genetic distances between populations of various geographical distances isolates and to reveal the character of genetic relationships between these populations. Therefore the STSM project aimed to find molecular markers that exhibit sufficient level of polymorphism to distinguish between different populations of *S. kraussei*.

Molecular markers which display a high frequency of within species polymorphism are required to distinguish between populations of the same species. Such markers are usually obtained from rapidly evolving regions of the genome such as microsatellite DNA or intron sequences. Because microsatellite sequences evolve rapidly, the regions flanking such sequences are highly variable even in closely related species, and microsatellite primers usually have to be developed independently for each species of interest. This can be very time consuming. On the other hand introns possess the advantage of being amplifieable within a broad range of taxa using one set of primers, because intron sequences are flanked by exons which provide conserved sites for PCR primers.

The host laboratory has developed PCR primers, which amplify intron sequences from 31 housekeeping gene in *Heterorhabditis*. These introns have been PCR amplified for all available species of *Heterorhabditis*, their DNA sequences have been obtained and the most polymorphic introns have been identified (Regeai and Burnell, personal communication). Five of these introns are highly polymorphic in their DNA sequences and some introns also display length polymorphisms. The STSM aim was to test whether some of these primers will amplify DNA introns from *S. kraussei* DNA.

Materials

Different populations of *Steinernema kraussei* used in the project are listed in table 1. Table 2 shows a list of available intron primers.

Table1: The list of the *Steinernema kraussei* strains used in the study:

Strain	Origin	Collected by
Ca 69	Canada	Z. Mracek
Ca 99	Canada	Z. Mracek
V.A. 6.4.	Bulgaria	M. Shishiniova
V.A. 7.2.	Bulgaria	M. Shishiniova
D1C	Czech Republic, Na Hrádecku	V. Puza
KR	Czech Republic, Ceské Budejovice	V. Puza
K	Czech Republic, Cejkovice	V. Puza
M	Czech Republic, Mydlovary	V. Puza
OHR	Czech Republic, Ohrada	V. Puza
P1	Czech Republic, Picina	V. Puza
PON	Czech Republic, Ponešice	V. Puza
SUB D3	Czech Republic, Na hrádecku	V. Puza
VC1	Czech Republic, Velký Cernoháj	V. Puza
VC2	Czech Republic, Velký Cernoháj	V. Puza
VES	Czech Republic, Veselí nad Lužnicí	V. Puza
ZR1	Czech Republic, Zliv	V. Puza

Table2: The list of primers used in the study.

Gene/Sequence name	**Primer name**
Serine/theronine protein kinase (ipp-5) / C09B8.1	ZBG 60826
Isocitratelyase (gei-7)/C05E4.9a	isocitratelyase
Zinc finger protein	zinc FP
Synaptosome associated protein family	SNAP –25
Casein kinase 1	Kin-19
Hypothetical protein (aspertic protease)	ASPERTICF
Calmodulin (cmd-1)	Calm
Protein required for normal RNA processing	HOA1148
Myosin heavy chain class II (unc-54)	Unc-54
Catalase	ZBG 60481
Protein containing an F-box motif	F-box
Phosphoenol pyruvate carboxykinase	ZBG 60468
Regulator of G protein signalling domain	RGS-2
Actin filament-coating protein tropomyosin	LEV-11
GTP binding protein like	GTP
F-actin capping protein beta subunit	ZBG 60859
Clatherine heavy chain (chc-1) / T20G5.1	ZBG 60830
Metalopeptidase / W08E12.7.	60452
GDI=1 GDP dissociation inhibitor	HOA1564
Voltage dependent anion selective channel protein / R05G6.7	FGE 60388

Experiments

At the beginning of the STSM, tentative experiments were carried out to learn and optimize methods employed. The DNA of Panagrolaimus (Af36, PS1579, PS443, PS1731) and Heterorhabditis (Hp88) strains and selected pairs of intron primers (Calm, Kin-19, Eft) were used.

DNA extraction from adult nematodes and larvae

DNA was extracted from giant females all of *S. kraussei* strains and from IJs of 4 selected strains using the DNeasy tissue kit (QIAGEN). Approximately 50 females or 2 ml suspension of IJs were used for a single extraction.

DNA extraction from single worms

DNA was extracted from giant females of several *S. kraussei* strains using a modification of the method developed by Qiu et al. (unpublished). Single adult females were placed into sterile 0.5 ml tube with 20 µl of worm lysis buffer (50 mM KCl, 10 mM tris pH 8.3, 2.5 mM $MgCl_2$, 0.45% NP40, 0.45% Tween 20, 0.01% gelatin and 60 µl / ml proteinase K) and crushed with pipette tip. The tube was frozen at –80°C for 10 minutes, incubated at 65 °C for 1 hour and then heated at 95°C for 10 minutes. Subsequently the tube was cooled on ice and centrifuged at 12000 rpm for 2 minutes.

RFLP of *S. kraussei* strains

In order to confirm species identification, PCR-RFLP analysis of rDNA ITS region of *S. kraussei* strains was carried out using three restriction enzymes: Hinf I, Alu I and Mbo I. The VC2 and Ca69 strains were excluded as they did not produce enough ITS PCR product.

Screening for intron polymorphism

Three geographically distant *S. kraussei* strains (Canadian Ca 69, Bulgarian V.A.6.4. and Czech KR) were selected for basic screening. One strain of *Heterorhabditis bacteriophora* strain (HP88) was used as control. PCR reactions with different primer pairs (table 1) were performed. The annealing temperature 51°C was used for all PCR reactions. Primer pairs which seemed to successfully amplify target product were then used in PCR reactions under different conditions (higher annealing temperatures, different concentrations of primers and DNA) in order to optimise the PCR conditions.

Results

No variation in digestion patterns of strains tested was observed. This confirms the morphological identification of all of the isolates in the study as *Steinernema kraussei*. RFLP patterns are shown in figure 1.

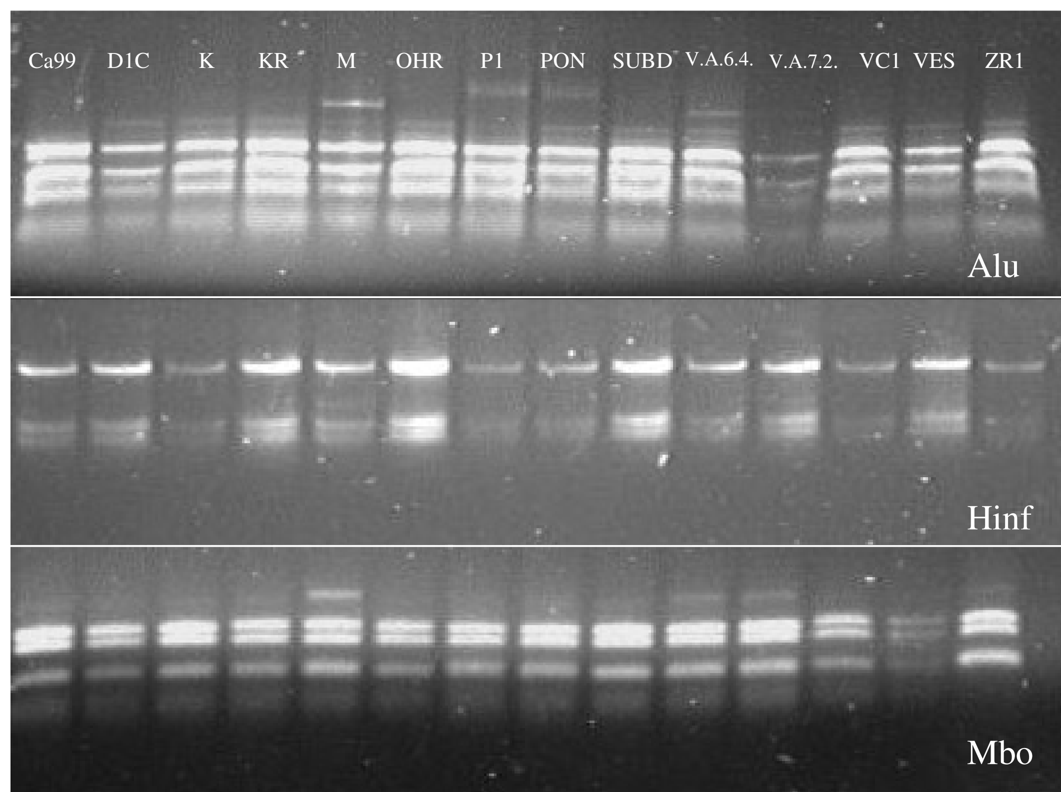

Fig.1: RFLPs yielded by digestion of *S. kraussei* strains by 3 restriction enzymes (AluI, HinfI and MboI).

Some of the tested intron primer pairs successfully amplified PCR products. PCR results achieved with the most successful primers are presented in figure 2. Table 3 shows the list of these primer pairs with additional details.

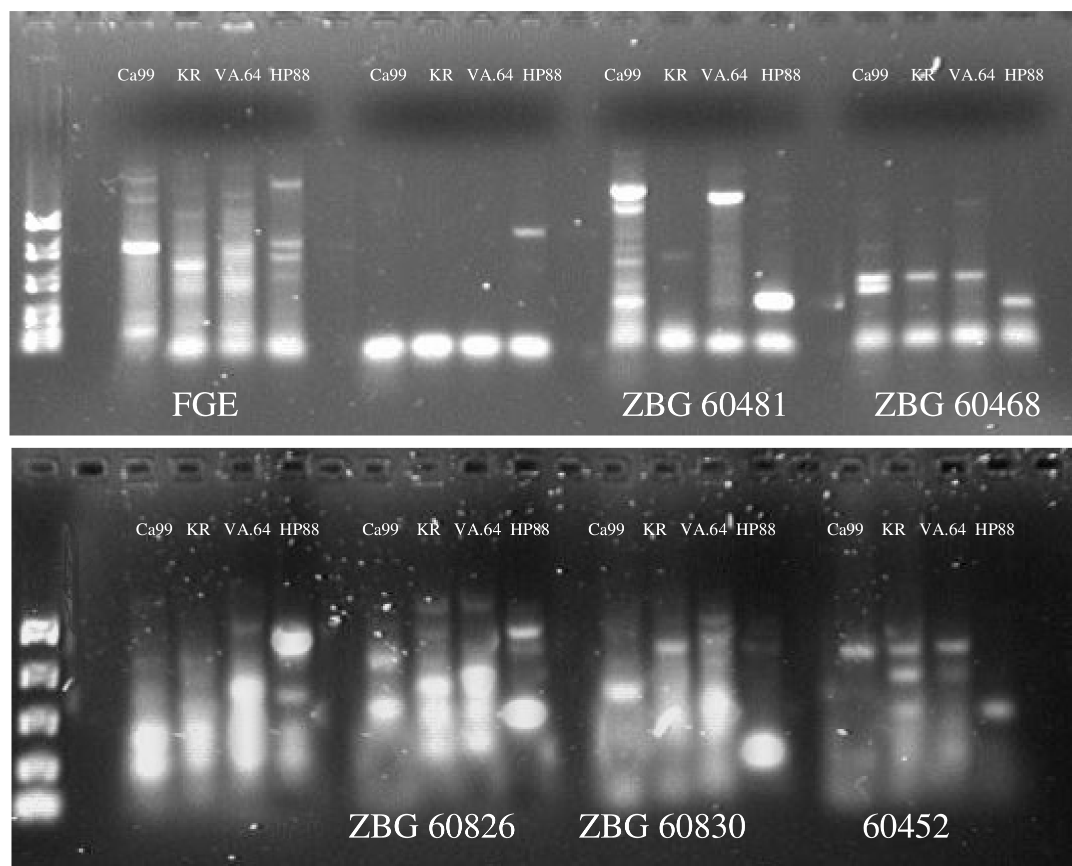

Fig.2: PCR products achieved using 6 selected primers. The ladder sizes are 766 bp, 500 bp, 300 bp, 150 bp, and 50 bp.

Table 3: Selected primer pairs.

Sequence name	Primer name
Voltage dependent anion selective channel protein	FGE 60388
Catalase	ZBG 60481
Phosphoenol pyruvate carboxykinase	ZBG 60468
Serine/theronine protein kinase (ipp-5)	ZBG 60826
Clatherine heavy chain (chc-1)	ZBG 60830
Metalopeptidase / W08E12.7.	60452

Conclusion and future prospects

Several intron primers (those listed in table 3) seem to be promising for the following population studies of *S. kraussei*. Some of them might even display length polymorphism in the target PCR product.

These primer pairs will be used in other set of PCR reactions in order to optimise PCR conditions for each primer pair. Finally, successfully amplified *S. kraussei* PCR products will be sequenced to confirm that the primers amplify the expected product. The sequence information will be also used to identify suitable restriction sites for carrying out restriction fragment length polymorphism (RFLP) analysis. Introns which display length polymorphisms

would be particularly valuable for future population studies, as they can be evaluated after PCR without an additional restriction digestion step.

Generally, in Maynooth I have received training and experience in basic molecular methods (extraction of DNA, gel electrophoresis, PCR, RFLP analysis) and I have identified primers which could distinguish between different populations of *S. kraussei*. These primers will be employed in my study of *S. kraussei* population biology.