COST 850
SHORT TERM SCIENTIFIC MISSION REPORT

On:
IDENTIFICATION OF EPN AND CROSS-BREEDING TESTS

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INTRODUCTION

During stay in the STSM host institution, Institute for Phytopathology, Department for Biotechnology and Biological control, Christian-Albrechts-University Kiel Raisdorf, Germany, my trainers and persons who gave great contributions to my studies were Dr. Ralf-Udo Ehlers, Dr. Arne Peters, Dr. Alper Susurluk, and Dr. Eustachio Tarasco.

The aim of the STSM were to gain knowledge of laboratory methods concerning morphological identification, and cross-breeding studies of entomopathogenic nematodes, as well as to improve my skills in using molecular biological techniques (PCR-RFLP) in EPN identification, particularly, working on nematodes from EU samples.

EU soil samples from six countries have been processed in Finland as part of the project MASTER, using trap insect technique in order to extract entomopathogenic nematodes.

STSM PROGRAM

1. Molecular biology methods

We processed 11 strains isolated from four EU counties, without previous morphological observation. DNA of all 11 strains was isolated, while for PCR and RFLP profiles only two, from mentioned 11 strains, were obtained. In PCR technique, the conditions for *Steinernema* species proceeding were used, according to the colour of dead larvae after nematodes infection (larva did not change the colour as the *Heterorhabditis* infected larvae do).

For one strain, obtained RFLP profile identified S.feltiae, while for another strain, obtained profile showed differences with other *Steinernema* species. After observing morphological characteristics, it is concluded that the strain, which had different RFLP profile, *is Heterorhabditis*, not *Steinernema.*

Furthermore, profiles of *Heterorhabditis* species were compared with obtained one; there were differences between obtained profile and profiles of known *Heterorhabditis species.*

The infection of *Galleria mellonella* larvae with the unidentified strain was repeated in order to measure photo luminescence. Measuring results showed no photoluminescence in infected larvae, although, by morphological characteristic, the strain is *Heterorhabditis*.

Further examinations are needed, as well as repeating of the same analyses to confirm the obtained data and to point out precise conclusions.

2. Morphological identification

I observed and learnt the main morphological characteristics and differences of different *Steinernema* and *Heterorhabditis* species by host institution's collection of permanent slides, prior to EU nematodes preparation for morphological identification.

Nematodes isolated from the EU soil samples were used in insect larvae infection. After 48 h of incubation, the dead larvae body was dissected in the Ringer's solution suspension, and adults were used for slide preparation for microscoping.

Permanent mounts were prepared by processing the strain that were unidentified with PCR-RFLP analysis. For permanent mounts preparation, method in the three different glycerol solutions was used. Permanent slides were prepared with adults and IJ, while with one part of IJ were processed by killing at 60 ºC in drop of water on the glass slide.

3. Cross-breeding

To obtain knowledge and practice, two different species (*S. feltiae* and *S. carpocapse)*, and two nematodes from the same specie (*S. feltiae)* were crossed.

IJ were placed in 10 x hyamine solutions for few minutes. On the sterile cover glass, drop of haemolymph was placed (obtained form surface-sterilised G. larvae in 95% ethanol). IJ were placed into drop of haemolymph. Then, cover glass were turned upside down and placed on concave slide, which was in sterile Petri dish on a filter paper saturated with sterile water. Petri dish is left to incubate at the room temperature. Evaluation was observed every day. After nematodes developed to adults, male and female were placed together. In one Petri dish male and female of *S. feltiae* and *S. carpocapsae* respectively, and in another Petri dish male and female of *S. feltiae* were placed together. Third day it was easy to distinguish female from male. After two days occurred copulation. In sample with two *S. feltiae* occurred progeny but not in sample with *S. feltiae* and *S. carpocapsae*.
Crosses should have sufficient replications to validate the results.

Besides learnt laboratory methods in the institute, we have intention scientifically collaborate in the future, as well as to publish the results of our work after completion of needed analyses.

Helsinki, 12.07.2004. Melita Zec-Vojinovic