

Short-Term Scientific Mission Report

REFERENCE: Short Term Scientific Mission, COST 850
Beneficiary: Lemma Ebssa, University of Hannover
Host: Michael Wilson, University of Aberdeen
Period: 20/07/2005 to 16/08/2005 (the dates are adjusted to available flight)
Place: Aberdeen (UK)
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A. Training covered

DNA extraction from animal tissue (i.e., nematode); PCR and gel-electrophoresis; Working with quantitative PCR (Real-Time PCR) along with the associated software.

The first two weeks were used to familiarize with the above training areas. The remaining time was used to carry out experiments planned before my arrival at the University of Aberdeen.

During the training period, DNA was extracted from different species of nematodes following QIAamp[®] DNA Mini Kit. Real-time PCR was used to amplify and quantify the extracted DNA (Fig 1). Then, the PCR products were separated and confirmed for their amplification using a gel-electrophoresis (Fig 2).

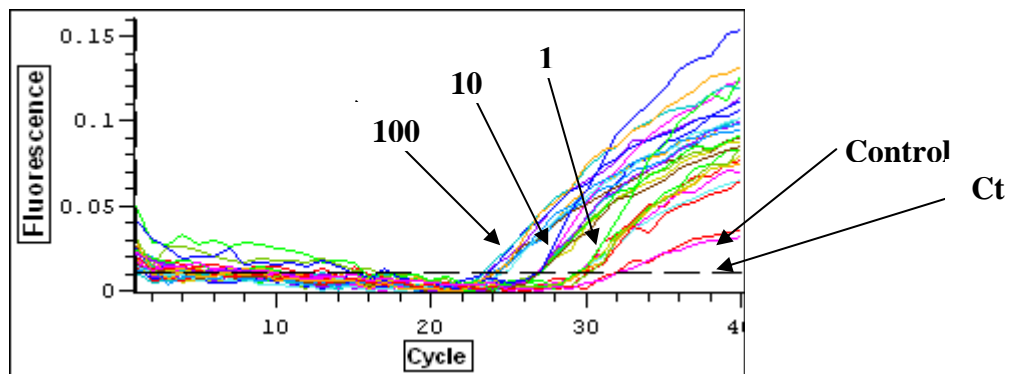


Fig 1. An example of quantification of *Phasmarhabditis hermaphrodita* juveniles (100, 10, or 1 individual nematode) per sample along with a negative control. Note: Ct is a threshold cycle.

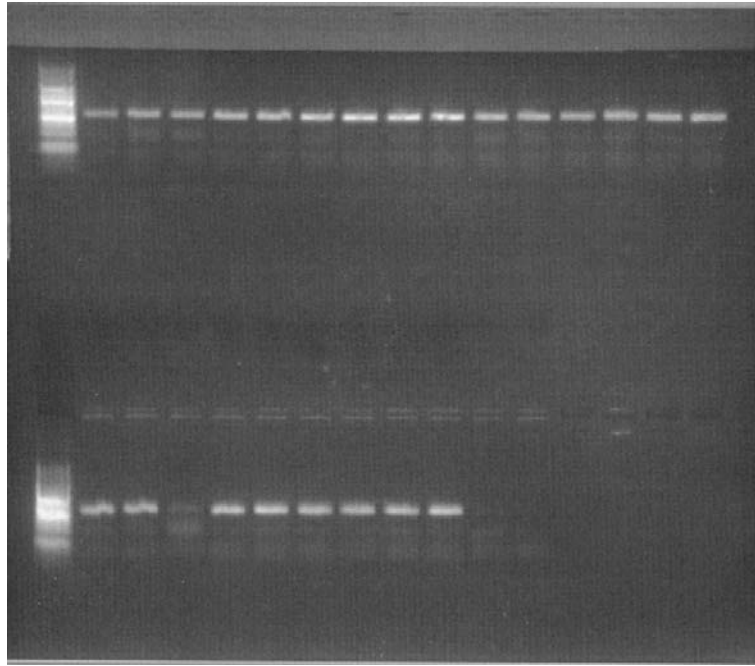


Fig 2. Gel-electrophoresis of PCR products

B. Experiments

1. Field experiment: Use of Real-time PCR for nematode persistence study

Fifty *Phasmarhabditis hermaphrodita* juveniles per cm² were applied to plots (55 cm x 75cm) using a watering can at an application rate of 1 litre tape water per plot. Application of the nematodes was followed by a post-irrigation of an additional litre of water to rinse watering can and to wash the nematodes down into the soil. The plots were arranged in two blocks in which two nematode-treated and one untreated plots were included in each block giving a total of four treated and two control plots. The control plots were irrigated with an equal amount of tape water to treat the plots. The plots were prepared from plastic boxes filled with soil. The nematodes were freshly ordered from Becker Underwood, UK and were only one week old. They were suspended in tape water and the quantification method described in Kaya and Stock (1997) was used to adjust the concentration to the required level just before the field application. From each plot soil samples were taken randomly from three spots of ca 2 cm depth and ca 4 cm diameter. Soils from all spots per plot were mixed and brought to lab. Soil samples were collected on the first day (day 0), 2, 4, 8, and 16 days after nematode application. Sampled spots in the plots were marked to avoid any re-sampling. Two sub-samples of 10 gram each were taken from each soil mixture. A

total DNA from the sub-samples was extracted following an UltraClean™ Mega Soil DNA Kit (Mo Bio Laboratories Inc.) (Fig 3). Shortly, the methodology is described as follows. Soil samples were added to a bead beating tube containing beads, lysis solution, bead solution and Inhibitor Removal Solution. The principal is to lyse the micro-organisms in general and the nematodes in this study in particular in the soil by a combination of heat, detergent, and mechanical force against specialized beads. The cellular components are lysed by mechanical action on a vortex. From the lysed cells, the released DNA is bound to a silica spin filter. The filter is washed, and the DNA is recovered in certified DNA-free Tris buffer. The isolated DNA was stored at 4 °C until use.



Fig. 3. UltraClean™ Mega Soil DNA Kit (Mo Bio Laboratories Inc.).

2. Laboratory experiment: How good is Real Time PCR to quantitate DNA from dead nematodes

A similar experiment as the one in the field was repeated in the lab except that the nematode-applied treatments were split into application of live or dead nematodes. The nematodes were killed by heat and cold shocks at 40 °C and on ice for 10 min in several intervals until the nematodes died. One gram of soil from experimental plots in the field was added to eppendorf tubes. Then, 50 live or dead *P. hermaphrodita* juveniles (suspended in 100 µl tap water) or tap water (as control) was pipetted onto the surface of the soil in the eppendorf tube. For a given DNA extraction time, four eppendorf tubes were used per treatment. DNA from the soil was extracted using an

UltraClean™ Soil DNA Kit (Mo Bio Laboratores Inc.) (Fig 4) which was similar to the UltraClean™ Mega Soil DNA Kit described above. The DNA in the soil was extracted on the same day of nematode application, 2, 4, 8, 16, (30, 60, 90 and 120) day after nematode application (For days in the bracket, DNA extraction will continue by the staff in Dr Wilson's lab). The eppendorfs with soil and nematodes were kept at 4 °C. The isolated DNA was also stored at 4 °C until used for amplification.



Fig 4. UltraClean™ Soil DNA Kit (Mo Bio Laboratores Inc.).

3. PCR analysis

In the DNA extraction, the whole DNA in the soil (including DNA of the nematodes that we applied and other living organisms in the soil) would be extracted. To amplify the 18S region of the genomic DNA of the nematode in this study, i.e., *P. hermaphrodita*, Forward and Reverse primers and probes suitable for Real Time PCR had been designed. Thus, theoretically, samples of DNA extracted from soils over different periods can be loaded to the Real Time PCR for amplification along with standard DNA solutions from a known number of nematodes. The primers and the probe will amplify mainly the 18S region region of the nematodes we are interested in due to their high specificity during their design. After amplification of the target region of the standards and the samples, usually after cycle 20 in Real Time PCR, a standard line (log quantity of nematodes vs. threshold cycle) can be calculated for the standards (Fig. 5). Using the same mathematical function of the standard curve, the quantity of the unknown number of nematodes in the samples can be estimated.

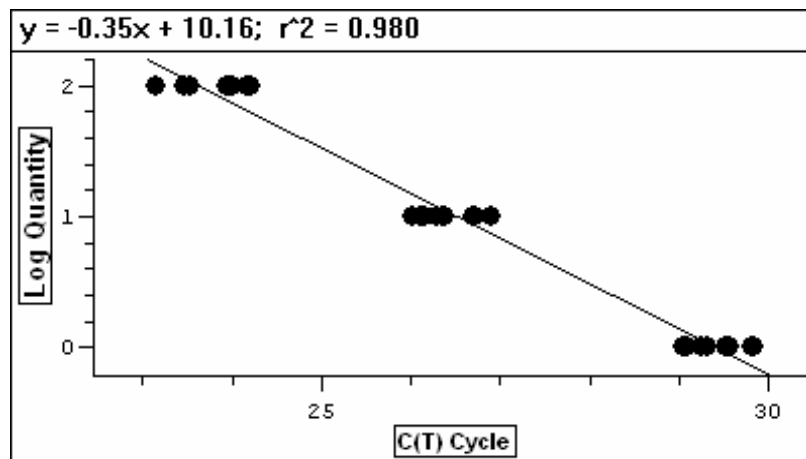


Fig 5. Mathematical function of log quantity of nematodes known number of nematodes (i.e., standards) vs. threshold cycle of DNA amplification.

Due to short period of time, all the results of PCR could not be obtained during my stay. Preliminary results showed that there was a low recovery of DNA from the soil using the kits described above. Further optimization of the procedures may be required. Currently, the staffs in University of Aberdeen and myself are working in this direction.

Conclusion

The scientific mission was very fruitful and I have gained an appreciable level of training during my short-term mission. Upon optimizing some of the protocols I used, the kits maybe used in soil DNA extraction in our future nematode quantification in field experiments. Furthermore, I am looking forward to collaborating with Dr Wilson's lab in my future research work. I would like to use this opportunity to thank Dr Mikael Wilson and the staff in his lab for sharing me their experiences and the COST office for financial support.