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(162) The use of PCR-HRM technique for detection of the quarantine nematode *Bursaphelenchus xylophilus*

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INTRODUCTION

High Resolution Melting Analysis (PCR-HRM) is a recently developed technique for fast, high-throughput post-PCR analysis of genetic mutations or variance in nucleic acid sequences. It enables researchers to detect rapidly and categorize genetic mutations, identify new genetic variants without sequencing, or determine the genetic variation in a population prior to sequencing (Pasay *et al.*, 2008).

We have examined this technique for detection and distinguishing of the quarantine pest, *B. xylophilus* from the morphologically and genetically most similar nematode *B. mucronatus*.

MATERIALS AND METHODS

Genomic DNA of *B. xylophilus* (isolate China) and *B. mucronatus* (isolate Wro-01) (about 100 nematodes) was isolated with QIAamp DNA Micro Kit (QIAGEN) according to the protocol provided by the manufacturer. For each species the isolation was conducted separately. The specific primers were designed from the ITS-1 region (forward: 5'- CGTGCAACGGTAAAGTCTGGGTTT-3' and reverse 5'- AATCCTACGCTCGCCAGAACGAAT-3') (Fig. 1). The PCR product was expected to be 112 bp in length. The PCR-HRM assays were performed with the use of Rotor-Gene Q Thermocycler (Qiagen). The obtained data was analysed according to the manufacturer's instructions.

A

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...|...|...|...|...|...|...|...|...|...|
      160   170   180   190   200
BmWro-01 CGCACGGAAG CCGAGAGGTG ACCGTGCAAC GGTAAAGTCT GGGTTCTAT
Bx       CGCATGGAAG CCGAGAGGCG ACCGTGCAAC GGTAAAGTCT GGGTTCTAC

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B

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...|...|...|...|...|...|...|...|...|...|
      260   270   280   290   300
BmWro-01 GCTGCTTGCC GATTCGTTCT GCGAGCGTA GGATTGAAAA GCCCGAGCGG
Bx       GCTGCTTGCC GATTCGTTCT GCGAGCGTA GGATTGAAAA GCCCGAGAGG

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Fig. 1. Diagrams indicating differences in sequence alignment of the forward primer (A) and reverse primer (B) between *B. xylophilus* and *B. mucronatus*.

RESULTS AND DISCUSSION

The study conducted with the designed primers allowed us to distinguish and identify two morphologically similar nematode species, *i.e.* *B. xylophilus* and *B. mucronatus*. Melting curve analysis of the reaction products showed the presence of a single amplification product for each of the performed reactions (Fig. 2). The graph shows mean values from four replicates of each reaction.

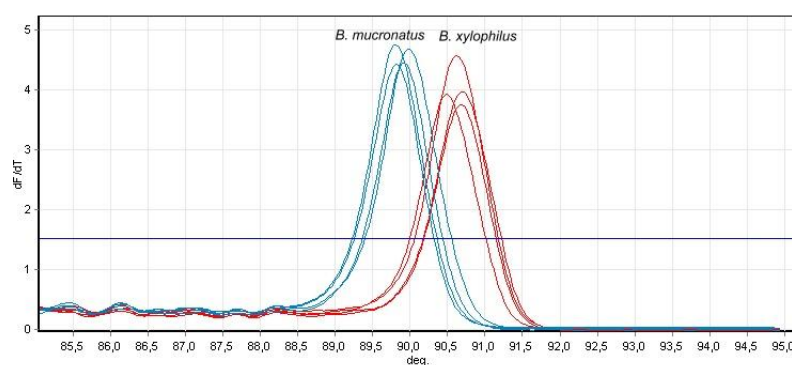


Fig. 2. Melting curves of PCR-HRM products: *B. xylophilus* and *B. mucronatus*. The vertical axis shows the fluorescence value (dF/dT), and the horizontal axis the value of the melting temperature (T_m) (°C).

The normalized DNA melting curves obtained in the HRM analysis differed from each other in denaturation temperature, as evidenced by a substantial shift of these curves in relation to each other. This indicates the differences between examined species in the composition of nucleotides within the amplified region of the genome. The analogous differences are also evident in the normalized differentiating graph (Fig. 3).

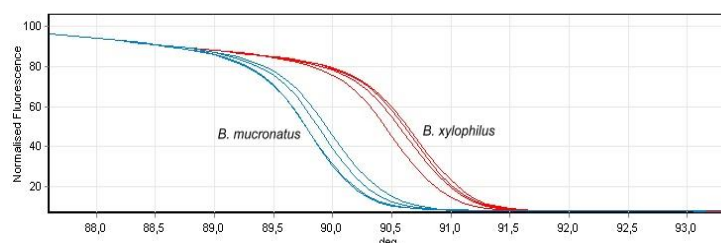


Fig. 3. Normalized differentiating graph of PCR-HRM products for: *B. xylophilus* and *B. mucronatus*.

Conducted study confirmed the high efficiency of the designed primers to distinguish the quarantine nematode *B. xylophilus* from the most closely related *B. mucronatus*. Since the PCR-HRM reaction allows for detection of single changes in nucleotides in tested of PCR products, it seems to be a very promising method for supporting identification decisions in the case of closely related species and isolates which could give ambiguous results in the real-time PCR reaction. Compared to the other molecular methods, PCR-HRM technique can be much simpler and less expensive way to identify the quarantine nematode *B. xylophilus*.

REFERENCES

- Pasay C, Arlian L, Morgan M, Vyszynski-Moher D, Rose A, Holt D, Walton S, McCarthy J. (2008). High-resolution melt analysis for the detection of a mutation associated with permethrin resistance in a population of scabies mites. *Medical and Veterinary Entomology* 22, 82–88.