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Specific Detection of *Vairimorpha* spp. in Lepidoptera by Multiplex Polymerase Chain Reaction

CHOI, JI-YOUNG[1,2]*, YEON-HO JE[2], JONG-GILL KIM[1], YOUNG-CHEOL CHOI[1], WON-TAE KIM[1], AND KEUN-YOUNG KIM[1]*

[1]*Department of Sericulture and Entomology, National Institute of Agricultural Science and Technology, R.D.A. Suwon 441-100, Korea*

[2]*Department of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Korea*

Objectives

We report the development of a multiplex PCR-based procedure for rapid, sensitive, and specific detection of entomopathgenic microspordia and differentiation of the genus *Nosema* and *Vairimorpha* and identification of the *Vairimorpha necatrix*.

Materials and Methods

1. Microsporidia

Unidentified microsporidia were obtained from *Pieris rapae* and *Artogeia rapae* larvae collected from an insectarium in Korea (Figs. 1, 2). All species were determined to be *Nosema* and *Vairimorpha* species by morphological features and DNA sequences of the V4 region of rRNA gene. Partial nucleotide sequences for the V4 region of ribosomal RNA gene of the microsporidia isolated from *P. rapae* and *Artogeia rapae*, were lodged in the GenBank database under Accession No. AF485270, AY311589, AY311590, and AY311592.

2. Primer design

The MSSR primer sets were designed based on the nucleotide sequences of the small subunit ribosomal RNA gene of *Nosems* sp. C01 Accession No. AF485270. VSSU primer set was designed according to the nucleotide sequences of the small subunit ribosomal RNA *Vairimorpha* sp. C21 Accession No. AY311592. Finally, VNAG primer was designed based on nucleotide sequences of actin gene of *V. necatrix* Accession No. AF031818. All primers sequences used in this study are given in Table 1.

3. Sensitivity of detection

In order to investigate the sensitivity of the PCR detection using the primers, serial ten-fold dilutions of the genomic DNA samples were prepared from the microsporidia spores. The reactions were prepared with genomic DNA, respectively. The PCR reaction was carried out as described above using MSSR primer set, and the products were electrophoretically separated on 1.0% agarose gel and stained with ethidium bromide.

Results and Discussion

Microsporidiosis is an infection caused by one of many species of the genus *Nosema*, *Vairimorpha*, *Thelohania*, *Pleistophora* and *Orthosomella* in Lepidoptera, the most common pathogenic genus of which

is *Nosema*. However, the number of infections by the recently identified genus *Vairimorphs* has increased, particularity in *Pieris rapae*. The various signs and symptoms associated with microsporidiosis in insects from obvious tissue manifestations to abnormal developmental and behavioral change. The two most infected insect tissues from which microsporidia have been reported are the fat body and midgut epithelium.

In this study we have demonstrated that PCR is a sensitive and specific method for diagnosing microsporidia infections in insect. Among the molecular techniques, PCR, restriction mapping and hybridization probes, PCR has been most widely employed for microsporidia diagnosis and epidemiologic studies.

The SSU rRNA gene sequences of many microsporidia have been elucidated and found to diverge greatly from other eukaryotes; the sequence is shorter and shares little homology with other eukaryotes. Thus, the SSU rRNA genes of the microsporidia possess characteristics amenable to molecular detection. The microsporidia SSU rRNA gene-specific primers we developed, successfully amplified 832 bp fragment from DNA preparations from purified microsporidia spores. Conversely, this primer pair did not amplify DNA preparations from uninfected larva, several other fungi and bacteria. Therefore, the MSSR primer sets exhibit specificity entomopathogenic microsporidia toward.

The genus *Vairimorpha* SSU rRNA gene-specific primers we developed, successfully amplified 570 bp fragment from genus *Vairimorpha* DNA. Conversely, this primer pair did not amplify DNA preparations from other genus microsporidia. Therefore, the VSSU primer set exhibits specificity genus *Vairimorpha* toward. The VNAG primer set, specific for the actin gene of *V. necatrix*, served as a positive control in multiplex PCR with the *V. necatrix*-specific primer set. These results suggest that SSU rRNA gene and actin gene are good candidates for the specific detection of genus *Vairimorpha* and *V. necatrix*, respectively.

A multiplex PCR system offers some advantages for the detection of genus *Vairimorpha* and *V. necatrix*, such as detecting coinfections simultaneously, simplifying diagnostic procedures, and saving labor time and costs. In this study, we used two sets of primer, one specific for *V. necatrix* and the other for genus *Vairimorpha*, as well as a third set that recognizes both. The products amplified from genus *Vairimorpha* with these primers were sufficiently specific to allow for the differentiation of genus *Nosema*.

The PCR assay developed in this study is expected to reduce the time to routine diagnosis. In conclusion, we have developed a multiplex PCR assay to detect entomopathogenic misrosporidia and to discriminate between genus *Nosema* and genus *Vairimorpha*. With the primer set used in this study, entomopathogenic microsporidia could be detected at a concentration of 10 spores per milliliter. Although more trials are needed concerning the detection of other *Vairimorpha* species in Lepidoptera, it is expected that the multiplex PCR assay with the primers we used will be a useful and valuable tool for the detection of the microsporidia.

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