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Characterization of an entomopathogenic *Nosema* sp. and secondary structure of its ribosomal RNA

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Insects in nearly all taxonomic orders are susceptible to microsporidia, but over half of the susceptible hosts occur in two orders, Lepidoptera and Diptera. To breed lepidopteran larvae on a large scale, the study of characterization and detection methods for entomopathogenic microsporidia must be carried out without delay. Therefore, this study was initiated to examine characteristics of entomopathogenic microsporidia isolated from *Pieris rapae*. In order to better understand the structural evolution of ribosomal RNA (rRNA) in microsporidia, rRNA gene was investigated in *Nosema* sp. C 01. Finally, multiplex PCR-based procedure was developed for rapid detection of microsporidia based on sequence of their rRNA gene of microsporidia. First, a microsporidium was isolated and characterized for its rRNA gene structure, spore morphology and pathogenicity. From the observation of the microsporidium by SEM and TEM, the endospores, exospores, nuclei, about 12-13 polar filament coils of the polar tube and posterior vacuoles were observed. The nucleotide sequence was determined for a portion of genomic DNA which spans the V4 variable region of the small subunit (SSU) rRNA gene. Comparison with the GenBank database for 11 other microsporidia species suggests that this isolate is most closely related to Nosema species. The pathogenicity against P. rapae was quantified by inoculating various doses of spores to the second instar larvae. Peroral inoculation at a dosage of 108 spores/ml resulted in the death of all larvae prior to adult eclosion, but at lower spore dosages of 10^4-10^5 spores/ml, many adults successfully emerged. The median lethal dose (LC_{50}) was determined to be 4.6 $\times 10^6$ spores/ml and the isolate was also transmitted transovarially to the progeny eggs at a frequency of 92%. Second, this study presented here for the first time the complete base

sequence (3,779 bp, GenBank Accession No. AY383655) of the rRNA gene of a lepidopteran-infecting microsporidium, *Nosema* sp. C 01. The SSU rRNA consists of 1,236 bp which is much shorter than a typical prokaryotic one. Its predicted secondary structure consists of a core (formed by 1st, 2nd, and 31st helices) and 4 branches (formed by 1st - 21st, 22nd - 30th, 32nd - 48th, and 49th - 50th helices) from the 5' end clockwise to the 3' end. The helices 10th, 11th, 18th, 37th, 43rd, 45th and 46th were missing. The large subunit ribosomal RNA (LSU rRNA) is also greatly reduced in length (2,506 bp). In its secondary structure, eleven hypervariable areas were shown and nine helices (B6, B7, B8, B14, B21, D5, E9, E15, and G5) are also missing. B7 - B9 and D4 helices can be used for taxonomic studies. Third, a multiplex polymerase chain reaction (PCR) was developed for the simultaneous detection and differentiation among *Vairimorpha* spp. and *Nosema* spp. and identification of *Vairimorpha necatrix* from Lepidoptera insects. Three sets of primers were selected from different genomic sequences to specifically amplify an 831 bp amplicon within the SSU rRNA gene, specific for both *Vairimorpha* spp. and *Nosema* spp. (MSSR primer); a 542 bp amplicon within the SSU rRNA gene, specific for *Vairimorpha* spp. (VSSU primer); and a 476 bp amplicon within the actin gene, specific for *Vairimorpha necatrix* (VNAG primer). Using the primers in conjunction (multiplex PCR) it was possible to detect and differentiate *Vairimorpha* spp. and *Nosema* spp. and to identify *V. necatrix*. The sensitivity of this PCR assay was approximately 10 spores per milliliter. The above-mentioned results suggest that microsporidial rRNA sequences and their secondary structure might contribute to their somewhat limited taxonomy based on morphology. In addition, the multiplex PCR is a sensitive, specific and rapid tool that can serve as a useful differential diagnostic tool for detecting *Nosema* spp. and *Vairimorpha* spp. in Lepidoptera.