学 位 論 文 要 旨

Studies on Two Alternaviruses, which Proposed to be a New Mycoviral Family—Alternaviridae, and Identification of a Novel Deltaflexivirus

新規マイコウイルス科－Alternaviridae 科を構成する 2 種類のアルタナウイルスの研究、及び新規デルタフレキシウイルスの同定

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Mycovirus was first discovered on cultivated mushrooms in 1962. Since then, more and more fungal viruses have been reported. In addition to infecting fungi, mycovirus-related viruses have been found on insects, plants, and oomycetes. The types of the genome of mycoviruses are double-stranded RNA, single-stranded RNA, circular single/double-stranded DNA, and single-stranded RNA reverse-transcribing.

The family name Alternaviridae was proposed in 2013 by a research group in the UK based on the paper by Aoki et al. (2009). Nowadays, we know that the fungal hosts of alternaviruses are *Alternaria* sp, *Aspergillus* spp. *Fusarium* spp., and *Diaporthe* sp. There are ten alternaviruses reported now. Alternaria alternata virus 1 (AaV1) was identified in the saprophytic fungus *A. alternata* strain EGS 35-193 (Aoki et al., 2009). AaV1 has four genomic double-stranded (ds) RNA segments (dsRNA1–4) packaged in isometric particles. The 3′ end of each positive strand within the particles is polyadenylated (36–50 nt), but the presence of a cap structure at each 5′ end was unknown.

In this study, I investigated that the dsRNA segments of alternaviruses have unique properties of terminal structures. The protein composition of the viral particle of alternaviruses and the purified viral particles of deltaflexivirus were also determined. It is expected to provide taxonomic criteria for virus taxonomy and to expand new knowledge in virology and molecular biology. Firstly, I characterized the AaV1 genome and found that it has unique features among the mycoviruses. The existence of cap structures at the 5′ ends of the AaV1 genomic dsRNAs was confirmed using RNA dot blots with anti-cap antibodies. Polyclonal antibodies against purified AaV1 particles specifically bound to an 82 kDa protein, suggesting that this protein is the major capsid component. Subsequent analysis of LC-MS/MS indicated that the AaV1 dsRNA3 segment encodes the major coat protein. And the smaller size proteins of empty AaV1 particles are also related to dsRNA3-encoded protein.

I also investigated the two kinds of defective AaV1 dsRNA2, which is 2,794 bp (844 aa) in length when intact, appeared in EGS 35-193 during subculturing, as confirmed by RT-PCR and northern hybridization. Sequence

analysis revealed that one of the two defective dsRNA2s contained a 231 bp deletion, while the other carried both the 231 bp deletion and an additional 465 bp deletion in the open reading frame (ORF). Both deletions occurred in-frame, resulting in 767 aa and 612 aa hypothetical proteins. The fungal isolates carrying virions with the defective dsRNA2s showed impaired growth and abnormal pigmentation. To the best of my knowledge, AaV1 is the first dsRNA virus that have both 5′cap and 3' poly (A) tail which is accompanied with complementary poly (U) structure at the ends of genomic segments and some isolates have defective dsRNA2s.

Secondly, I identified a novel dsRNA mycovirus—Diaporthe alternavirus 1 (DAV1) from the *Diaporthe* aff. *acuta* strain IbSTRPmp18001, isolated from a crown rot of strawberry. The isometric virions (ca. 35-40 nm in diameter, buoyant density: 1.349-1.374 g/cm^3) of DAV1 consist of four double-stranded RNA (dsRNA) segments and 79.3 kDa coat proteins. These four dsRNA segments are dsRNA1 (3.7 kbp), dsRNA2 (2.7 kbp), dsRNA3 (2.5 kbp), and dsRNA4 (1.7 kbp); each segment has a 5' cap structure and a 3′ poly (A: U) structure. Each of the four-dsRNA segments of DAV1 has a single ORF, dsRNA1 encodes an RNA-dependent RNA polymerase (RdRp), like other alternaviruses, the glycine residue is replaced by an alanine in the most conserved GDD motif. dsRNA3 encodes the coat protein. dsRNA2- and dsRNA4-encoded proteins are hypothetical proteins. The phylogenetic analysis of the amino acid sequence of RdRp indicates that DAV1 is classified as a member of Alternaviridae but has only about 30-40% sequence identity with other alternaviruses. A virus-free strain was obtained during subculture on the media. Comparisons of the hyphal morphologies and fungal growth between the DAV1-infected and the DAV1-free strains showed no significant differences, suggesting that DAV1 latently infects the host fungus. DAV1 is the first alternavirus found in *Diaporthe* sp. These results of alternaviruses may contribute to the fundamental molecular and biochemical characteristics and classification of the proposed Alternaviridae.

Finally, I also identified a novel deltaflexivirus which was isolated from *Fusarium oxysporum* fsp. *melonis*. The full-length viral genome was sequenced and the phylogenetic analysis was performed based on the replication proteins of the viruses. This novel virus is a positive ssRNA virus (8125 nt in length with five ORFs), classified into *Deltaflexiviridae*, named Fusarium deltaflexivirus 2 (FDFV2). In the purified virus suspension, isometric and filamentous particles were observed and were associated with the FDFV2 ORF4-encoded 18 kDa protein, which should be the major viral protein. To the best of my knowledge, this is the first report of particle and protein analysis observed in association with the deltaflexivirus. The results of 5'RACE showed that FDFV2 might have subgenomic RNAs to express the viral proteins. I will perform northern hybridization with riboprobes to confirm the organization of the subgenomes.