学位論文の要旨

Abstract of Thesis

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学位論文題目 Title of Thesis（学位論文題目が英語の場合は和訳を付記）

Novel mycoviruses isolated from *Rosellinia necatrix*
（白紋羽病菌から分離された新規マイコウイルス）

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Rosellinia necatrix is a soil-borne phytopathogen that causes white root rot in perennial plants worldwide. The fungus infects over 400 plant species and is very difficult to control by conventional control measures. Inspired by the success of biological control of the *Cryphonectria parasitica* by hypoviruses in Europe, Dr. Matsumoto's group started to search the field isolates of *R. necatrix* for double-stranded (ds) RNAs, which are considered to be mycovirus genomes or replication intermediates with virocontrol potential. Among these investigated isolates, approximately 20% strains were found to contain single or mixed dsRNAs. Since the first report on mycoreovirus 3 (MyRV3) in 2002, several novel viruses have been molecularly characterized, that include members of five previously (*Reoviridae*, *Partitiviridae*, *Totiviridae*) and newly established families (*Megabirnaviridae* and *Quadriviridae*). All *R. necatrix* viruses characterized thus far are dsRNA viruses, and no single-stranded (ss) RNA or DNA viruses have been found.

Here, we report a biological and molecular characterizations of three novel viruses infecting *R. necatrix*, which are designated as Rosellinia necatrix fusarivirus1 (RnFV1), yado-nushi virus 1 (YnV1) and yado-kari virus 1 (YkV1). RnFV1 was isolated from a field strain NW10, while the other two viruses were from a single fungal strain, W1032 where a unique virus/virus interplay occurred.

To investigate possible biological effects of RnFV1, two isogenic fungal strains, a virus-free and a virus-carrying strain, must be prepared. To this end, a recently developed technology using zinc ions allowed us to transfer RnFV1 to two mycelially incompatible *R. necatrix* strains. A biological comparison of the virus-free and -recipient isogenic fungal strains suggested that RnFV1 infects latently and thus has no potential as a virocontrol agent. The virus has an undivided positive-sense (+) RNA genome of 6286 nucleotides excluding a poly (A) tail. Both 5'-RACE and RLM-RACE were able to determine precisely the 5'-teminal sequences, which is 5'-UUUUU---AAAAG-3'. The genome possesses two non-overlapping open reading frames (ORFs): a large ORF1 that encodes polypeptides with RNA replication functions and a smaller ORF2 that encodes polypeptides of unknown function. A lack of coat protein genes was suggested by the

failure of virus particles from infected mycelia. No evidence was obtained by Northern analysis or classical 5′-RACE for the presence of subgenomic RNA for the downstream ORF. Sequence similarities were found in amino-acid sequence between RnFV1 putative proteins and counterparts of a previously reported mycovirus, Fusarium graminearum virus 1 (FgV1). Interestingly, several related sequences were detected by BLAST searches of independent transcriptome assembly databases one of which probably represents an entire virus genome. Phylogenetic analysis based on the conserved RNA-dependent RNA polymerase (RdRp) showed that RnFV1, FgV1, and these similar sequences are grouped in a cluster distinct from distantly related hypoviruses. It is proposed that a new taxonomic family termed *Fusariviridae* be created to include RnFV1 and FgV1.

YnV1 and YkV1 were isolated from field strain W1032, which shows a debilitation phenotype compared with virus-free isogenic strains. YnV1, which is present as a mixture of similar variants, has an undivided dsRNA genome of approximately 9.0 kilobase (kb). The genome contains two open reading frames (ORFs): ORF1 encoding polypeptides of capsid protein (CP) and ORF2 encoding polypeptides with RdRp function. Thus, the genome organization is similar to well-studied totiviruses, while the YnV1 genome is approximately twice as large as the totivirus genome. YnV1 is distantly related phylogenetically with dsRNA virus members of the family *Totiviridae*. Peptide mass fingerprinting (PMF) and N-terminal sequencing showed that CP initiates at the one third region of ORF1. YkV1 has a genome of approximately 6.3 kb containing one single large ORF. Phylogenetic analysis of the putative RdRp encoded by the large ORF showed that YkV1 is placed into a cluster comprising (+)ssRNA virus members of the family *Caliciviridae*. The *Caliciviridae* family accommodates human-infecting viruses such as noro-and sapoviruses. However, unlike for these viruses, a lack of CP gene was suggested by the sequence and PMF analyses for YkV1. Another interesting feature is virus genome variability of YnV1 and YkV1. Interestingly, at least three YnV1 strains and one defective RNA of YnV1 lacking a RdRp coding domain, were present in the original W1032 strain. Furthermore, during repeated subculturing of standard strain W97 transfected with W1032 virions, a defective RNA consisting of partial 5'- and 3'-terminal sequences of YkV1 appeared. This defective RNA will be examined as a foundation for vector development.

An interesting virus/virus interplay was observed in W1032. Immunological and molecular analyses revealed trans-encapsidation of not only YkV1 RNA but also RdRp by the major CP of the other virus, YnV1. Virion transfection assay and previous epidemiological data strongly suggest that YkV1 depends on YnV1 for viability, although it probably encodes functional RdRp. When a full-length cDNA of YkV1 was transformed into standard strain W97, this cDNA was infectious only in the presence of YnV1, confirming that YkV1 rely for its viability on YnV1. The comparison of accumulation of YnV1 genomic dsRNA between mycelia infected singly and doubly with YkV1 showed that YnV1 dsRNA was increased in fungal isolates infected by both viruses, suggesting that YkV1 benefits YnV1 replication and enhances production of YnV1 CP. I propose the term “RNA virophage” for the capsidless (+) RNA virus, YkV1, which highjacks CP of the dsRNA virus, YnV1, for the trans-encapsidation of its genome and RNA polymerase at the replication site. Instead, YkV1 enhances dsRNA and CP production of YnV1.

This study represents the first discovery of (+)ssRNA viruses of *R. necatrix* whose genomes have been thoroughly characterized. Another noteworthy revelation is the development of a reverse genetic system for the virus of *R. necatrix*. Lastly, this study has revealed a new virus life style challenging the virus rules and concepts: a unique mutualistic interaction between YnV1 and YkV1.

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