北海道大学　大学院農学院　修士論文発表会，2019年2月8日

Responses of mycoviruses to soil acidity in an arbuscular mycorrhizal fungus *Rhizophagus clarus*

応用生物科学専攻　生命分子化学講座　根圏制御学　Yu Duan

Mycoviruses are fungal viruses that possess, in most cases, a double-stranded or single-stranded RNA genome. Mycoviruses have been reported in several arbuscualr mycorrhizal fungi, and among them, one found in *Rhizophagus clarus* strain RF1 has a negative impact on the fungus, it reduced spore production and attenuated the growth promotive effect of the fungus on its host plant in acidic soil. On the other hand, another virus that belongs to the genus *Mitovirus*, *R. clarus* mitovirus 3 (RcMV3), increased its copy number in response to soil acidity, leading to the hypothesis that AM fungal viruses, or at least some of them, are associated with the acid tolerance of host fungus.

Lotus japonicus was inoculated with the acid tolerant AM fungus *R. clarus* strain CK001 isolated in Indonesia and grown at pH 3.5, 3.8, 4.2, and 4.9 in the mesh-bag separated culture system, by which fungal hyphae could be collected from the root-free hyphal compartment. Total RNA was extracted from extraradical hyphae, and mRNA was purified and subjected to 150-bp paired-end RNA-Seq. The sequence reads were *de novo* assembled, and in the contigs viral sequences were searched against the database with the Blastx algorithm. The viral sequences were assigned at the genus level based on protein domain of the viral replicase RNA-dependent RNA polymerase (RdRp). Viral abundances were compared by read mapping on the genomic sequences after normalizing to transcripts per kilobase million.

The BLASTX searches raised two putative mitoviral sequences that are 4.4 and 2.6 kb in length and likely to encode a full-length ORF of RdRp. Alignment of the amino acid sequences of the two with those of the other mitoviral RdRps indicated that the two sequences conserved the complete domain of Mitovirus RdRp (Pfam PF05919), by which 4.4- and 2.6-kb viruses were designated as *R. clarus* mitovirus 4 (RcMV4) and *R. clarus* mitovirus 5 (RcMV5), respectively. Phylogenetic analysis showed that the two viruses form two distinct clades, respectively, together with other mitoviruses recently found in AM fungi, but not with those in other fungi, suggesting their ancient origins. The abundance of RcMV4 in the extraradical hyphae of the host fungus was highest both at pH 3.8 and lowest at pH 4.2, whereas that of RcMV5 was constant along the pH gradient. These observations suggest that there is positive selection pressure on RcMV4 in acidic soil, but not on RcMV5.

In this study, two new mitoviruses were found in the Indonesian isolate of *R. clarus*, which were distinct compared with the other mitoviruses found in the different geographic isolates of the same species. The role of these viruses in acid-tolerance of the host fungus is unknown and to be elucidated via the establishment of virus-free lines.