2A-like protease activity is essential for replication and viability of yado-kari virus 1 hosted by yado-nushi virus 1

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Purpose: A unique mutualistic virus-virus interplay has recently been demonstrated in Rosellinia necatrix, where a positive sense, single-stranded RNA mycovirus, yado-kari virus 1 (YkV1) snatches the capsid protein (CP) from a co-infecting double-stranded RNA virus, yado-nushi virus 1 (YnV1) to encase its genome and RNA-directed RNA polymerase (RdRp). In return, YkV1 trans-enhances YnV1’s accumulation. We hypothesize that YkV1 utilizes YnV1’s CP as the replication site using its own RdRp. Interestingly, YkV1’s polyprotein contains a 2A-like sequence at its C-terminus. The picornavirus 2A oligopeptide has a conserved motif, DxExNPGP (where ‘x’ is any amino acid), and cleaves co-translationally between the G and P. Here, we examined whether 2A-mediated cleavage is essential for YkV1’s viability.

Methods: YkV1 mutants with amino acid substitutions in the 2A-like region were constructed and their replication competence and self-cleaving capabilities were examined in R. necatrix (harboring YnV1) and sf9 insect cells, respectively.

Results and conclusions: Mutants with alanine substitutions of the highly conserved sixth P and seventh G residues of 2A-like motif (DVEKNPGP) failed to replicate. Nevertheless, mutants retained replication competence when amino acids were altered at fifth and eighth residues. Insect cell-based protein expression showed a congruence between virus replication competence and 2A-like protease activity. These results further confirmed that complete cleavage at 2A-like motif is prerequisite for efficient replication of YkV1. Taken together with our previous results, this study indicates that YkV1, while depending on YnV1 CP for trans-encapsidation, utilizes its own RdRp that is likely functional only after being cleaved by the 2A-like protease.