Complete nucleotide sequences of genome segments 1 and 3 of Rosellinia anti-rot virus in the family Reoviridae

Brief Report

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Summary. The nucleotide sequences of genome segments 1 and 3 of Rosellinia anti-rot virus (RArV) from a hypovirulent isolate, W370, of the plant pathogen Rosellinia necatrix were determined. The complete nucleotide sequence of the genome segment 1 encoded a putative RNA-dependent RNA polymerase (RDRP). The deduced amino acid sequence of RDRP of RArV showed 29% identity with RDRPs of Colorado tick fever virus (CTFV) and European Eyach virus (EYAV) in the genus Coltivirus, and identities of 23–21% with members of the genera Fijivirus and Cypovirus. Both RArV and the Coltivirus member might have originated from a common virus ancestor.

Rosellinia anti-rot virus (RArV), a new member of the family Reoviridae, is isolated for the first time from a hypovirulent isolate, W370, of the plant pathogen Rosellinia necatrix Prillieux [15]. Morphological characterization of RArV and sequence analysis of most genome segments had suggested that RArV was presumably constitutive of a new genus of the family Reoviridae [11, 15]. However, the incomplete nature of the RNA sequence data of RArV did not clarify fully the genetic relationship among other members of the family Reoviridae. In this report, the complete nucleotide sequences of the genome

*The nucleotide sequence data reported in this paper for segment 1 and segment 3 of RArV will appear in the GenBank/EMBL/DDBJ nucleotide sequence databases under accession numbers AB102674 and AB102675, respectively.
segments 1 (S1) and 3 (S3) of RArV were determined. Phylogenetic relationship of RArV and other members of the family *Reoviridae* was discussed by comparison of amino acid sequence of the conserved polymerase protein deduced from segment 1.

The cDNA library derived from total dsRNAs of RArV was constructed as described before [15]. Clones specific to S1 or S3 were screened by Northern blot hybridization analysis [14, 15]. cDNA clones of the ends of S1 and S3 were determined by a 5′ RACE approach [7, 15]. Sequencing of the S1 and S3 specific cDNA clones and their subclones was performed using fluorescent labeled M13 forward and reverse primers, the thermo sequenase pre-mixed cycle sequencing kit (Amersham & Life Science) for Hitach SQ5500 DNA sequencer. Nucleotide data were compiled using the Genetyx program package (Software Development Co., Ltd).

The nucleotide sequence of the genome segment 1 was 4143 bases long and had a GC content of 47.8% with a long open reading frame (ORF). The deduced polypeptide (designed as VP1) contained 1360 amino acid residues (bases: 29–4110) with a predicted molecular mass of about 153 kDa. The search for sequence homologies using the NCBI BLAST 2.0 program (http://www3.ncbi.nlm.gov/)

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**Fig. 1.** Viral polymerase (RDRP) sequence analysis illustrated by a radial neighbor-jointing tree. Amino acid sequences derived from the nucleotide sequence of RNA polymerase of the relevant genome segment (segment 1 in each case, except for genome 4 of *Rice ragged stunt virus*, *Oryzavirus*; and segments 2 in *Cypovirus* and *Aquareovirus*), were aligned by using the Clustal W [13] and phylogenetic analyses were performed with MEGA version 2.1 [8] using the p-distance determination algorithm. Bootstrap values of 500 replications are indicated at the nodes (•) within each genus. Strains of representative viruses from ten genera of the family *Reoviridae* and their sequences used: *Orthoreovirus*: *Mammalian orthoreovirus*, subgroup 1. Lang strain (MRV-1) M24734; Jones strain (MRV-2) M31057; Dearing strain (MRV-3) M31058. *Orbivirus*: *African horse sickness virus*, serotype 9 (AHSV-9) U94887; *Bluetongue virus*, serotype 2 (BTB-2) L20508; serotype 10 (BTB-10) X12819; serotype 11 (BTB-11) L20445; serotype 13 (BTB-13) L20446; serotype 17 (BTB-17) L20447. *Rotavirus*: *Rotavirus A*, Bovine strain RF (BoRV-A/RF) J04346; bovine strain UK (BoRV-A/UK) X55444; Simian strain SA11 (SiRV-A/SA11) AF015955; Simian strain SA11-both (SiRV-A/SA11b) X16830; porcine strain Gottfried (PoRV-A/Go) M32805; *Rotavirus B*, human/murine rotavirus IDIR (Hu/MuRV-B/IDIR) M97203; *Rotavirus C*, Porcine Cowden strain (PoRV-C/Co) M74216. *Fijivirus*: *Nilaparvata lugens reovirus*, Izumo strain (NLRV-Iz) D49693; *Rice black-streaked dwarf virus* (RBSDV) AJ294757. *Phytoreovirus*: *Rice dwarf virus*, Chinese strain (RDV-Ch) U73201; *Rice dwarf virus*, strain H (RDV-H) D10222; *Rice dwarf virus*, strain A (RDV-A) D90198; *Oryzavirus*: *Rice ragged stunt virus*, Thai strain (RRSV-Th) U66714. *Coltivirus*: *Colorado tick fever virus* strain Florio, (CTFV-Fl) AF133428; European *Eyach virus* strain Fr578 (EYAV-Fr578) AF282467. *Seadornavirus*: *Banna virus* (BAV-Inf423) AF133430; *Kadijipiro virus* (KDV-Ja7075) AF133429. *Cypovirus*: *Bombyx mori cypovirus* 1 (BmCPV-1) AF323782; *Lymnantria dispar cypovirus* 1 (LdCPV-1) AF389463; *Lymnantria dispar cypovirus* 14 (LdCPV-14) AF389452; *Trichoplusia ni cypovirus* 15 (TnCPV-15) AF291683. *Aquareovirus*: *Grass carp reovirus* (GCRV) AF260512; *Golden shiner reovirus* (GSRV) AF403399; *Chum salmon reovirus* (CSV) AF418295.