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Sequence analysis of 12 genome segments of mud crab reovirus (MCRV)

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ABSTRACT

Mud crab reovirus (MCRV) is the causative agent of a serious disease with high mortality in cultured mud crab (*Scylla serrata*). This study sequenced and analyzed 12 genome segments of MCRV. The 12 genome segments had a total length of 24.464 kb, showing a total G+C content of 41.29% and predicted 15 ORFs. Sequence analysis showed that the majority of MCRV genes shared low homology with the counterpart genes of other reoviruses, e.g., the amino acid identity of RNA-dependent RNA polymerase (RdRp) was lower than 13.0% compared to the RdRp sequences of other reoviruses. Nucleotide and amino acid sequences of RdRp and capping enzyme suggested MCRV as a single group. Further genome-based phylogenetical analysis of conserved termini and reovirus polymerase motif indicates that this MCRV belongs to a new genus of the Reoviridae family, tentatively named as *Crabreovirus*.

Introduction

The mud crab, *Scylla serrata*, is an economically important marine species cultured in China; its production exceeded 113 thousand metric tons in 2008 (FAO, 2010). Mud crab reovirus (MCRV) is the causative agent of a "sleeping disease" (SD) with about 70% mortality, severely damaging the crab cultures in China. MCRV infects the connective tissue cells of the hepatopancreas, gills, and intestines, and multiplies in the cytoplasm. The complete viral particle of MCRV is icosahedral, non-enveloped, and 70 nm in diameter. No obvious spike structures are present on the surface of its virions (Weng et al., 2007).

Reoviruses are composed of double-stranded RNA (dsRNA) with 9, 10, 11, or 12 linear genome segments (Attoui et al., 2005a; Mertens et al., 2005). They have been isolated from a wide range of host species, including mammals, birds, reptiles, fish, crustaceans, insects, arachnids, plants, and fungi. According to the 8th report of the International Committee on Taxonomy of Viruses (ICTV), the family Reoviridae is classified into 12 genera that include 69 species (Mertens et al., 2005). In the years since the last ICTV report, three new Reoviridae genera, *Mimoreovirus*, *Cardoreovirus*, and *Dinovernavirus*, have been described (Attoui et al., 2006a: http://www.ictvonline.org/virusTaxonomy.asp?version=2009), rendering this family 15 distinct genera with total 84 species.

Since the first report of a crab virus by Vago (1966), many outbreaks of reovirus-infected crab diseases have been described. In addition to MCRV, these reoviruses include *Carcinus mediterraneus* W2 virus (CcRV-W2) from shore crabs, *C. mediterraneus*, (Mari and Bonami, 1988), *Macropipus depurator* P virus (MdRV-P) from Mediterranean swimming crabs, *M. depurator* (Bonami, 1973) and *Eriocheir sinensis* reovirus 905 (EsRV905), from Chinese mitten crab, *E. sinensis* (Zhang et al., 2004). EsRV905 has been partially sequenced and was determined to a new genus and species (Zhang et al., 2004). Based on histopathological properties, MCRV exhibits similar properties to MdRV-P and CcRV-W2. In addition, the pattern of dsRNA upon polyacrylamide gel electrophoresis revealed that MCRV (1/5/6(7)) closely resembled MdRV-P and CcRV-W2 (both 1/5/6), but was distinct from EsRV905 (3/4/2/3) (Mari and Bonami, 1988; Montanie et al., 1993; Zhang et al., 2004).

To date, the number of sequenced Reoviridae genomes is well above 100. However, much less is known about the genomes of the crab-originating reoviruses MdRV-P, CcRV-W2, and EsRV905. Both MdRV-P and CcRV-W2 have not been sequenced while EsRV905 has been only partially sequenced (Mari and Bonami, 1988; Montanie et al., 1993; Zhang et al., 2004). In this report, we describe for the first time the sequences of 12 genome segments of MCRV. Phylogenetic analysis determined that MCRV is a distinctive species of Reoviridae, suggesting that it should be assigned to a new genus.

Results

MCRV genome sequences

dsRNAs were isolated from purified virions of MCRV (Fig. 2A) and analyzed by polyacrylamide gel electrophoresis (PAGE). The result of

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PAGE showed there were 12 segments in the genome of MCRV with a 1/5/6 electrophoretic pattern (Fig. 1, lane T1). Full length sequences of the 12 MCRV segments were obtained and deposited in GenBank with accession numbers HQ414127–HQ414138 (Table 1). These 12 segments had a total length of 24.464 kb with a total G + C content of 41.29%. Northern blot analysis revealed that all clones were specifically hybridized, suggesting that each segment was genetically unique (Fig. 1, lane S1–S12).

Open reading frame (ORF) prediction of the MCRV genome by ORFfinder (http://www.ncbi.nlm.nih.gov/projects/gorf/) showed that each segment contained one large ORF (Table 1). Besides these 12 ORFs, three small ORFs were predicted in downstream of segment 4 (1720–2382) and segment 11 (858–992 and 961–1113). However, we were unable to confirm protein products of these three ORFs by TNT translation system.

Proteins of MCRV viral particles

Purified virions of MCRV were analyzed by SDS-PAGE (Fig. 2B). The result revealed there were 7 bands in the protein gels; of these, 6 bands ranged from 7 to 170 kDa and 1 band was approximately 200 kDa. As the position at 7 kD seems to be the bottom of the gel (notice there is no density in the lane below 7 kD), it is unclear whether this corresponds to a protein, proteins, or degradation products.

Analysis of non-coding regions of MCRV genome segments

Analysis of 5′ and 3′ non-coding regions (NCR) of MCRV genome showed that there are conserved nucleotides at 5′ termini and 3′ termini. For each segment of MCRV, the positive strand 5′-end was AUAAAUU (segment 1–2, segment 4–9 and segment 12), AUAAAUA (segment 3 and 10) or AUAAACU (segment 11). The positive strand 3′-end was GAUCAACGAU (segment 1–4, segment 6, 7, 9, 11 and 12), GAACAACGAU (segment 5 and 8) or GGUAAACUAU (segment 10). In sum, the consensus of MCRV termini sequences is 5′-AUAAA$^{U}/_{C}^{A}/_{U}$——$G^{A}/_{G}^{A}/_{U}^{A}/_{C}AAC^{G}/_{U}$AU-3′.

Comparing the conserved termini of MCRV segments with those of other reoviruses revealed that five nucleotides (AUAAA) in the 5′ termini were conserved between RRSV (Rice ragged stunt virus, *Oryzavirus*) and MCRV, and the last four nucleotides ($C^{G}/_{U}$AU) in the 3′ termini of MCRV were conserved with those ($^{U}/_{C}$GAU) of phytoreoviruses (RDV: Rice dwarf virus, HvRV: *Homalodisca vitripennis* reovirus, RGDV: Rice gall dwarf virus) (Table 2). Specially, we compared the conserved NCR sequences of MCRV with the NCR sequences of segment 1 of EsRV905 and found that only two nucleotides (GA) in the 3′ termini were conserved between MCRV and EsRV905.

As in the seadornaviruses, Rotavirus A, Rotavirus B (*Rotavirus*) and MpRV (*Mimoreovirus*), the first and last two nucleotides of all 12 genome segments of MCRV are inverted complements (AU and AU). This is distinct from RRSV, phytoreoviruses and Rotavirus C, where the first and last nucleotides in each segment are non-complementary, and distinct from EsRV905, which shows mono-nucleotide complementary. A folding analysis of positive strands of MCRV segments is performed by the software RNAStructure 5.0. The folding result predicted that 5′ and 3′ NCRs of 12 MCRV segments are able to be base-paired, forming panhandle structures (Fig. 3).

Conserved terminal sequences have been reported for all members of the Reoviridae, which may be involved in recognition signals for the viral transcriptase and replicase functions. These sequences may also be essential for selection and incorporation of the RNAs into the nascent progeny particles (Mertens et al., 2005). Imperfect inverted complements of positive strand 5′ and 3′ NCR of reovirus RNA have been found in many cases as well. It has been suggested that the complementary nature of sequences in the 5′ and 3′ NCRs is involved in regulation of RNA function, like translation, replication, or packaging (Anzola et al., 1987; Chen and Patton, 1998; Patton, 2001; Stenger et al., 2009; Theron and Nel, 1997).

Comparison of MCRV amino acid sequence with other Reoviridae members

Sequence comparisons were completed using BLASTp. All predicted polypeptides of MCRV were compared to the total gene collection of Reoviridae using BLASTp alignment. Alignment revealed some identities between MCRV and other reoviruses (Table 3).

VP1

VP1, encoded by MCRV segment 1, shared partial identities with RNA-dependent-RNA-polymerases (RdRps) of other reoviruses, specifically VP1 of the genus *Seadornavirus* (BAV, 21% in positions 706–1053; KDV, 20% in positions 373–1051; LNV, 19% in positions 452–1044), *Cardoreovirus* (EsRV905, 20% in positions 456–1080), and VP2 of

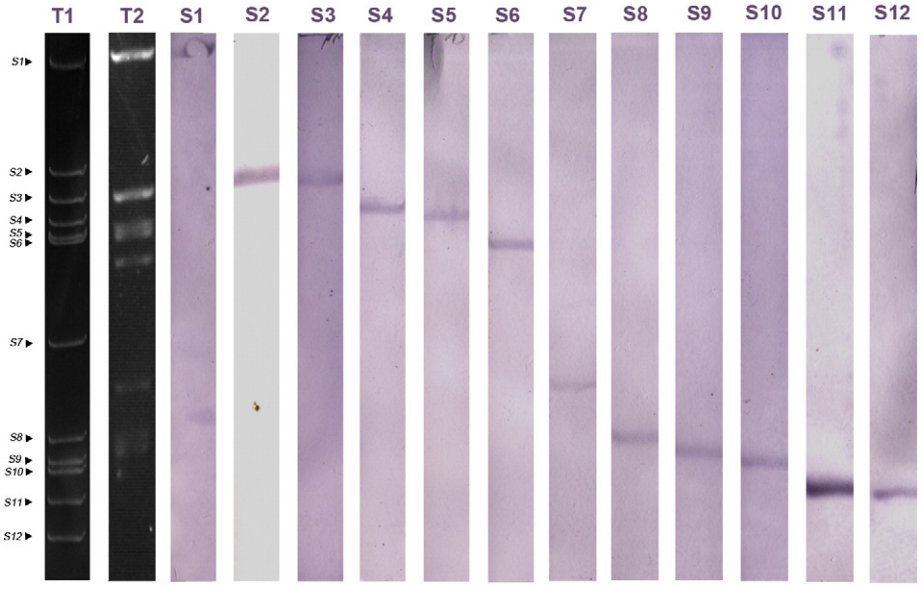

Fig. 1. Northern blot analysis to confirm sequences obtained from cDNA clones. Total dsRNA of MCRV was separated by 1% AGE (T2), transferred to nylon membranes, and hybridized with DIG-labeled cDNA clones prepared from the designed primers. T1 is the total dsRNA of MCRV separated by 15% PAGE.