Arch Virol (2016) 161:1385–1387
DOI 10.1007/s00705-016-2754-6

ANNOTATED SEQUENCE RECORD

Genomic characterization and evolution analysis of a mutant reovirus isolated from grass carp in Anhui

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Received: 13 October 2015 / Accepted: 2 January 2016 / Published online: 13 January 2016

Grass carp fingerlings and yearlings are vulnerable to grass carp reovirus (GCRV), leading to a mortality rate of more than 80 %. A virulent reovirus, tentatively named GCRV-AH528, was isolated from a diseased grass carp in Anhui Province, China, in 2014. The isolated virus could be propagated in *Ctenopharyngodon idellus* kidney cells. However, the typical cytopathic effects of aquareovirus infection were not observed with this strain. Intraperitoneal injection with the isolated virus led to hemorrhage similar to the observed clinical symptoms. This confirmed that GCRV-AH528 was a pathogenic strain. Its genome contained 11 double-stranded RNA segments (S1-S11) ranging from 1,027 to 3,925 bp (accession numbers KR180368 to KR180378), and encoding 12 proteins instead of 11 proteins (Table 1). All segments had conserved terminal nucleotides, with GUAA$^{U}/_{C}$U at the 5′ end and U$^{U}/_{G}$CAUC at the 3′ end. Compared with registered GCRV strains [1–3], the GCRV-AH528 S1 segment had lost three continuous nucleotides (TAT) at nt 701-702, leading to the loss of a tyrosine, and the S4 segment exhibited two ORFs encoding two proteins, indicating that GCRV-AH528 was distinct from any known GCRV and was a mutant reovirus. Sequence comparisons showed that GCRV-AH528 possessed only seven proteins that were homologous to those of the first isolate, GCRV-873 from 1983 (18.1 %-47.0 % sequence identity), but eight that were homologous to members of the genus *Orthoreovirus* (13.9 %-45.2 % identity). A phylogenetic tree based on RdRp revealed that GCRV-AH528 clustered with members of the genus *Aquareovirus* and had genetic affinity with to members of the genus *Orthoreovirus* (Fig. 1). This study will help in recognizing and understanding the molecular epidemiology, genetic diversity, and evolutionary relationships of GCRV.

Electronic supplementary material The online version of this article (doi:10.1007/s00705-016-2754-6) contains supplementary material, which is available to authorized users.

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Table 1 Characteristics of GCRV-AH528 genome segments

Segment/nt	AT (%)	GC (%)	CDS	Protein/aa	Protein size (kDa)	Isoelectric point (pI)	Conserved terminal sequences
S1/3925	50.11	49.89	18-3899	VP1/1293	143.61	6.00	5′-GUAACU UUCAUC-3′
S2/3867	49.63	50.37	16-3834	VP2/1272	142.38	8.86	5′-GUAAUU UUCAUC-3′
S3/3753	49.48	50.52	16-3714	VP3/1232	135.75	5.39	5′-GUAAUU UUCAUC-3′
S4/2265	48.43	51.57	25-255 302-2176	NS9&NS69/76&624	8.52 68.65	11.00 5.81	5′-GUAUUU UUCAUC-3′
S5/2230	49.64	50.36	13-2193	VP5/726	80.60	6.68	5′-GUAACU UUCAUC-3′
S6/2028	48.42	51.58	41-1993	VP4/650	68.43	5.73	5′-GUAAUU UUCAUC-3′
S7/1604	51.50	48.50	18-1556	VP56/512	56.06	5.04	5′-GUAAUU UUCAUC-3′
S8/1560	54.23	45.77	48-1133	VP41/361	41.25	8.91	5′-GUAAUU UGCAUC-3′
S9/1320	49.92	50.08	23-1279	VP6/418	47.95	8.13	5′-GUAACU UUCAUC-3′
S10/1124	50.27	49.73	25-1062	NS38/354	38.39	5.52	5′-GUAAUU UUCAUC-3′
S11/1027	50.54	49.46	35-967	VP35/310	35.49	6.88	5′-GUAAUU UUCAUC-3′
Average	50.20	49.80					5′-GUAA$^{U}/_{C}$U U$^{U}/_{G}$CAUC-3′

Incomplete sequences: nt, nucleotide; CDS, coding DNA sequence; aa, amino acid

Table 2 Sequences used for phylogenetic analysis

Subfamily	Genus	Virus strain	Accession number
Spinareovirinae	***Orthoreovirus***	Baboon orthoreovirus (BRV)	YP_004769548.1
		Avian orthoreovirus (ARV)	YP_004226522.1
		Avian orthoreovirus D20/99 (ARV-D20/99)	AHL21593.1
		Mammalian orthoreovirus 2 (MRV-2)	ABG49449.1
		Mammalian orthoreovirus 3 (MRV-3)	ABP48913.1
	Aquareovirus	**Grass carp reovirus AH528 (GCRV-AH528)**	**KR180369**
		Grass carp reovirus 873 (GCRV873)	AAG10436.1
		Golden shiner reovirus (GSRV)	AAM92745.1
		American grass carp reovirus (AGCRV)	ABV01040.1
		Chum salmon reovirus (CHSRV)	AAL31497.1
		Grass carp reovirus 104 (GCRV104)	AFG73673.1
	Coltivirus	Colorado tick fever virus (CTFV)	AAK00595.1
		Eyach virus (EV)	AAM18342.1
	Mycoreovirus	Mycoreovirus 1 (MYRV-1)	YP_001936004.1
	Fijivirus	Mal de Rio Cuarto virus (MDRCV)	AAO73182.1
		Nilaparvata lugens reovirus (NLRV)	NP_619776.1
	Oryzavirus	Rice ragged stunt virus Thai (RRSV-Thai)	NP_620541.1

Table 2 continued

Subfamily	Genus	Virus strain	Accession number
	Dinovernavirus	Aedes pseudoscutellaris reovirus (APRV)	YP_443936.1
	Cypovirus	Heliothis armigera cypovirus 14 (HACPV-14)	ABB51571.1
		Bombyx mori cypovirus 1 (BMCPV-1)	AAK20302.1
		Dendrolimus punctatus cypovirus 1 (DPCPV-1)	AAN46860.1
Sedoreovirinae	***Cardoreovirus***	Eriocheir sinensis reovirus (ESRV)	AAT11887.1
	Orbivirus	African horse sickness virus (AHSV)	AAC40994.1
		Epizootic hemorrhagic disease virus (EHDV)	AEM06328.1
	Seadornavirus	Banna virus (BV)	AHF50196.1
		Kadipiro virus (KV)	AAF78850.1
	Rotavirus	Simian rotavirus (SiRV)	P22678.1
		Porcine rotavirus (PoRV)	Q85036.1
	Phytoreovirus	Rice gall dwarf virus (RGDV)	YP_001111373.1
		Rice dwarf virus (RDV)	BAA01074.1
		Homalodisca vitripennis reovirus (HVRV)	ACO37232.1

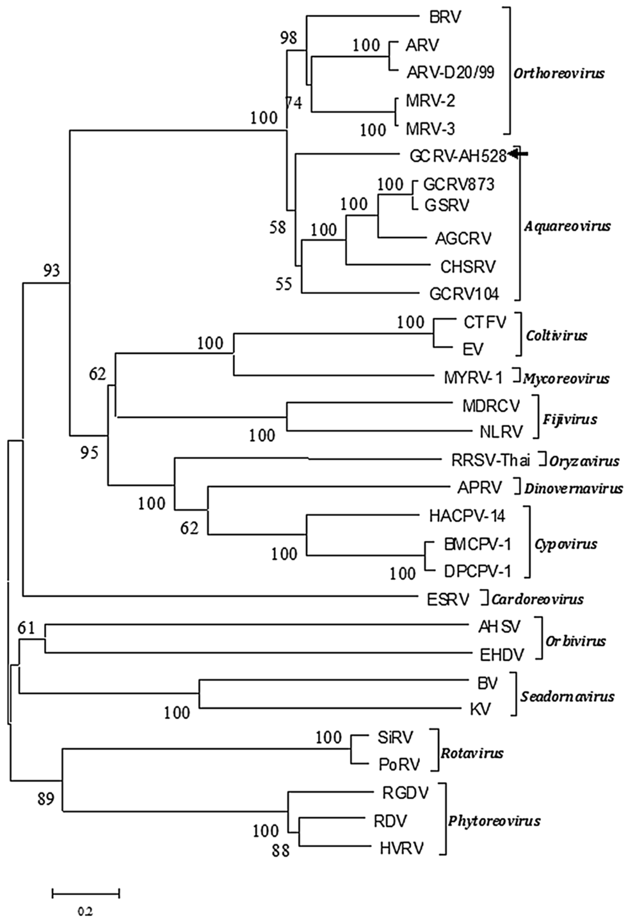

Fig. 1 Phylogenetic analysis based on amino acid sequences of RdRp from members of the family *Reoviridae*. The arrow indicates the position of GCRV-AH528. The phylogenetic tree was constructed by the neighbor-joining method using 1000 bootstrap replications. The abbreviations and RdRp GenBank accession numbers are presented in Table 2

Acknowledgments This work was supported by the Youth Fund of Anhui Academy of Agricultural Sciences (14B0531), the Scientific and Technological Innovation Fund of Anhui Academy of Agricultural Sciences (13C0506) and the Earmarked Fund for China Agriculture Research System (CARS-46).

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