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Virology 339 (2005) 81 – 100

VIROLOGY

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Complete genome sequence analysis of an iridovirus isolated from the orange-spotted grouper, *Epinephelus coioides*

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Received 25 February 2005; returned to author for revision 9 March 2005; accepted 11 May 2005
Available online 20 June 2005

Abstract

Orange-spotted grouper iridovirus (OSGIV) was the causative agent of serious systemic diseases with high mortality in the cultured orange-spotted grouper, *Epinephelus coioides.* Here we report the complete genome sequence of OSGIV. The OSGIV genome consists of 112,636 bp with a G + C content of 54%. 121 putative open reading frames (ORF) were identified with coding capacities for polypeptides varying from 40 to 1168 amino acids. The majority of OSGIV shared homologies to other iridovirus genes. Phylogenetic analysis of the major capsid protein, ATPase, cytosine DNA methyl transferase and DNA polymerase indicated that OSGIV was closely related to infectious spleen and kidney necrosis virus (ISKNV) and rock bream iridovirus (RBIV), but differed from lymphocytisvirus and ranavirus. The determination of the genome of OSGIV will facilitate a better understanding of the molecular mechanism underlying the pathogenesis of the OSGIV and may provide useful information to develop diagnosis method and strategies to control outbreak of OSGIV.

Keywords: OSGIV; Iridoviruses; Genome; Sequence analysis

Introduction

Iridoviruses are icosahedral cytoplasmic DNA virus that can infect invertebrates and poikilothermic vertebrates including the insects, fishes, amphibians, and reptiles (Williams, 1996). The viral genomes are both circularly permuted and terminally redundant which is a unique feature among eukaryotic virus genomes (Darai et al., 1983, 1985; Delius et al., 1984; Goorha and Murti, 1982). Additionally, the iridoviruses infect vertebrates that have highly methylated genomes (Darai et al., 1983; Tidona and Darai, 1997; Willis and Granoff, 1980). Currently, the entire genomes of nine iridoviruses have been completely sequenced. These viruses include lymphocystis disease virus 1 (LCDV-1, the type species of the genus *Lymphocystivirus*; Tidona and Darai, 1997; accession no. L63545), infectious spleen and kidney necrosis virus (ISKNV; He et al., 2001; accession no. AF371960), *Chilo* iridescent virus (CIV, the type species of the genus *Iridovirus*; Jakob et al., 2001; accession no. AF303741), tiger frog virus (TFV; He et al., 2002; accession no. AF389451), *Ambystoma tigrinum* virus (ATV; Jancovich et al., 2003; accession no. AY150217), lymphocystis disease virus isolated in China (LCDV-C; Zhang et al., 2004; accession no. AY380826), frog virus 3 (FV3, the type species of the genus *Ranavirus*; Tan et al., 2004; accession no. AY548484), Singapore grouper iridovirus (SGIV; Song et al., 2004; accession no. AY521625), and rock bream iridovirus (RBIV; Do et al., 2004; accession no. AY532606). Most of these viruses can infect low vertebrates except CIV, which is isolated from insects.

Based on the Seventh Report of the International Committee on Taxonomy of Virus (ICTV), the family Iridoviridae has been subdivided into four genera, including *Iridovirus*, *Chloriridovirus*, *Ranavirus*, and *Lymphocystisvirus* (van Regenmortel et al., 1999). Another type of iridoviruses from affected fish, which belong to neither lymphocystivirus nor ranavirus, can cause enlargement of cells in many tissues, especially in the spleen and kidney of

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0042-6822/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2005.05.021

fish. He et al. (2001) tentatively referred them as cell hypertrophy iridoviruses. In 2003, Chinchar et al. proposed a new genus, *Megalocystivirus*, to the International Committee on Taxonomy of Virus (ICTV). The proposed classification system of the family Iridoviridae included *Iridovirus*, *Chloriridovirus*, *Ranavirus*, *Lymphocystisvirus*, and *Megalocystivirus* (personal communication).

In recent years, megalocystiviruses have attracted much research attention because of their ecological and economic impact on wild and cultured fishes. Megalocystiviruses are well-known causative agents of many serious systemic diseases occurred in economically important freshwater and marine fish worldwide. Fishes infected by the megalocystivirus include the red sea bream, *Pagrus major* (Inouye et al., 1992); sea bass, *Lateolabrax* sp. (Nakajima and Sorimachi, 1995); brown-spot grouper, *Epinephelus tauvina* (Chua et al., 1994); Malabar grouper, *E. malabaricus* (Danayadol et al., 1996); angelfish, *Pterophyllum scalare* (Rodge et al., 1997); grouper, *Epinephelus* sp. (Chou et al., 1998); tilapia, *Oreochromis niloticus* (McGrogan et al., 1998); mandarin fish, *Siniperca chuatsi* (He et al., 2000); African lampeye, *Aplocheilichthys normani* (Sudthongkong et al., 2001); dwarf gourami, *Colisa lalia* (Sudthongkong et al., 2002); red drum, *Sciaenop socellata* (Weng et al., 2002); rock bream, *Oplegnathus fasciatus* (Jung and Oh, 2000); large yellow croaker, *Larimichthys crocea* (Chen et al., 2003); and turbot, *Scophthalmus maximus* (Shi et al., 2004).

With the rapid development of grouper culture, outbreaks of viral diseases occurred frequently in cultured orange-spotted grouper (*Epinephelus coioides*) in the culture farms of Guangdong Province, China. The causative agent was confirmed to be an iridovirus named orange-spotted grouper iridovirus (OSGIV). OSGIV is closely related to ISKNV, RBIV, and red sea bream iridovirus (RSIV) based on morphology, histopathology, epidemiology, and some nucleotide sequences information. As the disease was important to the orange-spotted grouper cultures, we have performed sequence analysis and molecular characterization of the OSGIV complete genome. We also performed phylogenetic analysis of the OSGIV proteins with that of other iridoviruses and discussed the taxonomic position of OSGIV.

Results and discussion

Determination of the viral genomic DNA sequence

Because no reliable cell lines could be used for the propagation and isolation of OSGIV, PCR was performed using primers of ISKNV to detect/identify diseased grouper. Sequencing of the PCR products revealed that the major capsid protein (MCP), ribonucleotide reductase small chain (RNRS), and cytosine DNA methyl transferase (DMet) of OSGIV shared 95%, 94%, and 95% identities to those of ISKNV at the nucleotide level (data not shown). We then developed a PCR approach to amplify OSGIV genome with the primers from nucleotide sequence of ISKNV (AF371960). The amplified PCR products were about 1000–1200 bp in length. Moreover, large numbers of overlapping primer pairs were designed to fill gaps and to confirm the sequence. The PCR products were purified and the DNA sequences were determined by a PCR sequencing kit (Applied Biosystems, Inc.). With this procedure, about 6× coverage of OSGIV genome sequence was accomplished.

The OSGIV genome contained a double-stranded DNA consisting of 112,636 bp with a G + C content of 54%. Among the sequenced iridoviruses, the size of the OSGIV genome was similar to that of ISKNV (111,362 bp) (He et al., 2001), RBIV (112,080 bp) (Do et al., 2004), FV3 (105,903 bp) (Tan et al., 2004), TFV (105,057 bp) (He et al., 2002), ATV (106,332 bp) (Jancovich et al., 2003), and LCDV-1 (102,653 bp) (Tidona and Darai, 1997), and slightly smaller than that of SGIV (140,131 bp) (Song et al., 2004) and LCDV-C (186,250 bp) (Zhang et al., 2004), but a much smaller than that of the invertebrate iridovirus, CIV (212,482 bp) (Jakob et al., 2001). The G + C content of the OSGIV genome was similar to those of ISKNV (54.8%), RBIV (53%), FV3 (55%), TFV (55%), ATV (54%), and SGIV (48.64%), but much higher than that of LCDV-1 (29.1%), LCDV-C (27.25%), and CIV (28.6%) (Table 1).

The OSGIV genome also contained numerous short direct, inverted, and palindromic repetitive sequences. A highly direct repetitive region was identified at position 24187 to 24642 bp in the OSGIV genome, which was also

Table 1
Summary of genomic information for 10 sequenced iridoviruses

Virus	Genus	Genome size (bp)	G + C content (%)	No. of ORFs	ORF size (aa)	Year determined	Accession no.
OSGIV	Unassigned	112636	54	121	40–1168	2004	AY894343
RBIV	Unassigned	112080	53	118	50–1253	2004	AY532606
ISKNV	Unassigned	111362	54.8	124	40–1208	2001	AF371960
FV3	*Ranavirus*	105903	55	98	50–1293	2004	AY548484
TFV	*Ranavirus*	105057	55	106	40–1294	2002	AF389451
ATV	*Ranavirus*	106332	54	96	32–1294	2003	AY150217
SGIV	*Ranavirus*	140131	48.64	162	41–1268	2004	AY521625
LCDV-1	*Lymphocystivirus*	102653	29.1	195	40–1199	1997	L63545
LCDV-C	*Lymphocystivirus*	186247	27.25	240	40–1193	2004	AY380826
CIV	*Iridovirus*	212482	28.6	468	40–2432	2001	AF303741