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Organ distribution of red sea bream iridovirus (RSIV) DNA in asymptomatic yearling and fingerling rock bream (*Oplegnathus fasciatus*) and effects of water temperature on transition of RSIV into acute phase

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Abstract

Most diagnostic studies of red sea bream iridovirus (RSIV) disease in fish have been conducted on the acutely infected fish, and spleen has been used commonly as a target organ for diagnosis. However, little information about persistent RSIV infection is available, and the distribution of RSIV in organs and tissues in persistently infected fish has not been systematically determined. In the present study, organ distribution of RSIV in asymptomatic carriers of yearling and fingerling rock bream was analyzed using PCR, and transition possibility of RSIV into acute phase in both age groups by elevating water temperature was investigated. RSIV DNA was predominantly detected in heart, stomach, intestine, muscle, eye and gill by nested-PCR from asymptomatic yearling rock bream. Strikingly, the detection frequency of RSIV DNA in spleen was very low. In fingerling rock bream, consistent with the result of yearling, the frequency of RSIV DNA detection in spleen was low. Fingerling rock bream showed a high cumulative mortality by water temperature elevation, and RSIV DNA was detected by primary PCR from dead fish. In yearling rock bream, however, there were no mortality and no detection of RSIV DNA by primary PCR during water temperature elevation period.

Keywords: RSIV; Persistent infection; Organ distribution; Rock bream; Temperature

1. Introduction

Iridoviruses are large, icosahedral, cytoplasmic DNA viruses, and infect a diversity of vertebrate and invertebrate hosts (Williams, 1996). Systemic iridoviruses in fish are important economically because of their association with large-scale mortality in aquaculture systems. Since 1990, outbreaks of red sea bream iridovirus (RSIV) disease have resulted in high mortalities in cultured red sea bream, *Pagrus major*, in Japan (Inouye et al., 1992). In Korea, high mortalities by RSIV infections in net-caged rock bream, *Oplegnathus fasciatus*, occurred firstly at 1998. Since then, RSIV disease has been the major culprit of mass mortality of

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rock bream in Korea (Sohn et al., 2000; Jung and Oh, 2000; Kim et al., 2002).

A number of diagnostic methods, including cell culture (Nakajima and Sorimachi, 1994), immunofluorescence (Nakajima et al., 1998) and polymerase chain reaction (PCR) (Miyata et al., 1997; Kurita et al., 1998; Oshima et al., 1998), have been developed for detecting RSIV in fish showing acute symptoms. However, little information is available on the diagnosis of persistent RSIV infection in fish. Persistent viral infection can generate sporadic outbreaks in infected fish and may result in shedding and transmission of infectious virus, leading to disease in susceptible fish. The increase in trade and movement of fish amplifies the potential for spread of iridoviruses through latently infected fish. Therefore, detection of iridoviruses in latently asymptomatic fish is important to prevent spreading of infected fish and to know epizootiological causes of iridoviral diseases.

The typical histopathological features of acute RSIV infections are splenomegaly and the presence of enlarged basophilic cells in the spleen, gill, kidney, heart and liver (Inouye et al., 1992; Jung and Oh, 2000; Wang et al., 2003). The most frequently used organ for diagnosis of RSIV infection was spleen. However, as there is no information on the organ distribution of RSIV in asymptomatic, persistent infection states, it is questionable whether the spleen is the suitable target for diagnosis of RSIV in asymptomatic carriers as in acutely infected fish.

In the present study, organ distribution of RSIV in asymptomatic carriers of yearling and fingerling rock bream was analyzed using PCR, and transition possibility of RSIV into acute phase in both age groups by elevating water temperature was investigated.

2. Materials and methods

2.1. Fish

Fingerling (about 3–4 g in body weight) rock bream, *O. fasciatus*, were obtained from a hatchery. Water temperature of the hatchery was 18–19 °C at the sampling time. Yearling (about 120–130 g in body weight) rock bream were sampled from a net-pen farm located on the southern coast of Korea, and water temperature at the sampling time was 16–18 °C. The farm had experienced RSIV disease last year. At the day of fish arrival in the laboratory, 5 yearlings and 10 fingerlings were sampled randomly to analysis organ distribution of RSIV. The other fish were acclimated for 2 weeks at 18 °C prior to the experiment of water temperature elevation.

2.2. Polymerase chain reaction (PCR)

Fish were anaesthetized with MS222 (Sigma Co., St Louis, MO, USA) and peripheral blood was collected from the tail vein with heparinized syringes. Leucocytes were isolated from the blood by Percoll (Sigma) gradient centrifugation. Spleen, kidney, liver, heart, stomach, intestine, brain, muscle, eye and gill were removed from the fish. In fingerling, each organ of 2 fish was pooled. Genomic DNA was isolated from approximately 50 mg of each organ using Accuprep® Genomic DNA Extraction Kit (Bioneer Co., Daejeon, Korea).

Two oligonucleotide primers, PF (5′-ATGTCTG-CAATCTCAGGTG-3′) and PR (5′-TTACAGGA-TAGGGAAGCCTGC-3′), were designed to amplify the major capsid protein (MCP) ORF of an iridovirus isolated from rock bream (GenBank accession number, AY532612), and the expected size of amplification product was 1362-bp. The PCR amplification procedure was carried out in a 20 μl reaction mixture containing 10 μl of PCR premix taq (Takara, Japan), 1 μl (10 pM) of each primer, 2 μl DNA template, and 6 μl of distilled water. The reaction was performed for 1 cycle of 3 min at 95 °C, 30 cycles of 30 s at 95 °C, 30 s at 65 °C, 30 s at 72 °C, with a final extension step of 7 min at 72 °C using an automated thermal cycler (iCycler, BioRad Inc., USA). Oligonucleotide primers, NF (5′-CACCGCAACGTGCAAAGCAA-3′) and NR (5′-TTGACTGCAATAACGACCAGTTCAAAC-3′), were used for nested-PCR, and the expected size of amplification c was 369-bp. DNA template of the nested-PCR was 1/100 dilution of the first PCR products, and the reaction condition was as described above. The amplified PCR product was cloned into a PCR 2.1-TOPO plasmid using the TOPO TA Cloning® Kit (Invitrogen, CA, USA), and the recombinant plasmid was purified using an Accuprep® Plasmid Extraction Kit (Bioneer) for sequencing.

2.3. Effects of water temperature elevation on transition of RSIV into acute phase

Fingerling and yearling rock bream were divided into 2 groups of 50 fish in 50 l aquaria and 2 groups of 25 fish in 400 l aquaria, respectively. Water temperature of one tank in each age group was elevated from 18 to 26 °C by increment of 2 °C per day. The fish in the other tanks were kept at 18 °C throughout the experiment. Fish that died during the experiment were deep-frozen at −70 °C until PCR analysis. At 21 d post-temperature elevation, fish were sampled randomly (10 fingerling and 5 yearling) from each tank, and were analyzed the presence