한국어병학회지 제 22 권 제 2 호 (2009)
J. Fish Pathol., 22(2) : 173 ~ 177 (2009)

- Note -

Comparison of lymphocystis disease virus proteins between marine and freshwater fish

Mosharrof Hossain, Sung-Ju Jung, Wi-Sik Kim, Seok-Ryel Kim* and Myung-Joo Oh†

Division of Food Science and Aqualife Medicine, Chonnam National University, Yeosu-550-749, Korea
**Pathology division, National Fisheries Research and Development Institute, Busan 619-902, Korea*

Lymphocystis disease virus (LCDV) was detected from olive flounder *Paralichthys olivaceus*, painted glass fish *Chanda baculis*, gourami *Trichogaster leeri* and rockfish *Sebastes schlegeli*, and proteins of the viruses were compared. The major capsid protein (MCP) gene-specific primer sets successfully amplified approximately 1300 bp nucleotides from the olive flounder and 600 bp nucleotides from painted glass fish, gourami and rockfish isolates, respectively. In western blotting analysis using anti-LCDV mouse polyclonal serum, major antigenic proteins had 21, 26, 45, 50, 80, 110 and 120 kDa in olive flounder, 26, 47 and 80 kDa in painted glass fish, 26, 46, 80 and 92 kDa in gourami, 26, 44, 49, 80 and 105 in rockfish, respectively. All the marine and freshwater isolates showed only common antigens of approximately 26 kDa and 80 kDa. These results suggest that antigenic protein profiles of LCDVs may vary depending upon fish species.

Key words: Lymphocystis disease virus, Anti-LCDV serum, Viral protein, Western blot

Lymphocystis disease virus (LCDV) is the causative agent of lymphocystis disease developing a tumor like cluster composed of hypertrophied fibroblast cells with cytoplasmic inclusions (Wolf, 1998). The tumors are usually found from the skin, fins and oral regions both in the marine and freshwater fishes worldwide. Lymphocystis disease affects more than 125 different wild and cultured fish species, causing important economic losses globally. In Korea, lymphocystis disease is the common fish disease, especially in aquaculture of olive flounder *Paralichthys olivaceus*, rockfish *Sebastes schlegeli* and sea bass *Lateolabrax* sp. (Kitamura *et al*., 2006a, 2006b). Additionally, in aquarist shops, the disease has been observed in ornamental fish species.

From the first report of the electron microscopic observation (Walker, 1962), great advances have been made in LCDV studies such as morphology, detection method and genome (Zwillenberg and wolf, 1968; Darai *et al*., 1983; Tidona and Darai, 1997; Tidona *et al*., 1998; Webby and Kalmakoff, 1998; Zhang *et al*., 2004; Cano *et al*., 2006; Kitamura *et al*., 2006a, 2006b). However, few researchers have reported on the protein profiles of LCDV (Flugel *et al*., 1982; Schnitzler and Darai, 1993; Garcia-Rosado *et al*., 2004). In the present study, LCDV from marine fish (olive flounder and rockfish) and freshwater ornamental fish (gourami, *Trichogaster leeri*, and painted glassfish, *Chanda baculis*) was detected, and proteins of the viruses were compared.

Materials and Methods

Fish sampling

The lymphocystis disease-affected freshwater ornamental fish, gourami and painted glass fish

†Corresponding Author : Myung-Joo Oh, Tel : 82-061-659-3173
Fax : 82-061-659-3173, E-mail : ohmj@chonnam.ac.kr

were bought from the local ornamental pet shop (Table 1). The lymphocystis disease-affected marine fish, olive flounder and rockfish were sampled from the commercial culture farms in Yeosu, southern part of Korea (Table 1).

PCR detection of LCDV

The lymphocystis cell clusters were removed from the fins and skin of diseased fish using a blade, then washed with hank's balanced salt solution (HBSS), and homogenized with 9-fold dilution of TE buffer (10mM Tris-HCl, 1mM EDTA, pH 7.4). After separating cell debris by centrifugation 2000 × g for 10 min at 4°C, 200 μℓ of supernatant of each samples were mixed with 20 μℓ aliquot of proteinase K (TaKaRa, Japan) in each tubes and incubate 45 min at 60°C. The DNA was extracted from all the samples by phenol-chloroform solution, and was subjected to PCR for major capsid protein (MCP) gene of LCDV with two primer sets, LCC-F&R (F-5′-CAA GTG TTA CTA GCG CTT T-3′ & R-5′-ATC CCA TTG AAC CGT TCT-3′) and LCDVs F&R (F-5′-GTA ATC CAT ACT TGH ACR TC-3′ & R-5′-YTG GTT CAG TAA ATT ACC RG-3′). The two primer sets were designed on the basis of MCP gene sequence information according to Kitamura *et al*. (2006b). The target sizes of the primer sets, LCC-F&R and LCDVs F&R are 1347 and 609 bp, respectively. A 20 μℓ mixture solution including DNA template of each samples in PreMix tube (Bioneer, Korea) was used for PCR amplification using a GeneAmp 2400 thermal cycler (Perkin Elmer, USA) with 35 cycles (95°C for 1 min, 54°C for 1 min, and 72°C for 1 min). The PCR product was analyzed in 2% agarose gel containing ethidium bromide and visualized under UV light.

Virus purification

For the virus purification, the PCR positive samples of each isolates were weighted and homogenized in TE buffer as described above. The homogenates were sonicated three times. To separate the debris, the homogenates was centrifuged at 500 × g for 20 min at 4°C. Then the supernatant was centrifuged again at 2000 × g for 20 min at 4°C. The supernatant was collected and stored at -20°C for overnight, Virus was pelleted down from the

Table 1. List of sampled fish species and LCDV detection by PCR

Fish species	Sampling sites	Sampling periods	Organ used for PCR	PCR positive /Total
Flounder (*Paralichthys olivaceus*)	Commercial farms, Yeosu	2006 (June-July)	Skin & fins	12/12
Rockfish (*Sebastes schlegeli*)	Commercial farms, Yeosu	2006 (June-July)	Fins	9/10
Gourami (*Trichogaster leeri*)	Pet shop, Yeosu	2006 (April -July)	Fins	6/9
Painted glass fish (*Chanda baculis*)	Pet shop, Yeosu	2006 (April-July)	Fins	4/7

stored supernatant by ultracentrifugation at 21,000 rpm for 120 min at 4°C and re-suspended in 2 ml final volume of TE buffer. The suspension was loaded onto pre-formed gradients made up of 20 to 50% (w/v) sucrose, and centrifuged at 25,000 rpm for 120 min at 4°C. Resulting bands were collected carefully and mixed with TE buffer and ultracentrifuged again at 25,000 rpm for 90 min at 4°C. Finally, virus pellets were re-suspended in 200 $\mu\ell$ of TE buffer and stored at -80°C for further use.

Western blotting

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis for the purified virus was performed according to Laemmli (1970). The purified viruses of each isolates were dissolved in loading buffer (0.5mM Tris-HCl, 2% SDS, 5% 2-mercaptoethanol, 0.1% bromophenol blue, 10% glycerol), boiled at 100°C for 5 min and applied onto the 4.5% stacking gel and viral proteins were separated by 12% SDS-PAGE. For western blotting, LCDV viral proteins in the gel were transferred to nitrocellulose membrane using a transfer apparatus for 45 min at 75 V (Pharmacia, USA). The membrane was blocked overnight with 10% skim milk in T-PBS (150 mM NaCl, 10 mM Tris-HCl pH 7.5, 0.05% Tween-20). Then, anti-LCDV mouse polyclonal antiserum diluted 1:200 was applied and incubated for 1 h at room temperature. After 10 min's rinsing 3 times in T-PBS, the membrane was incubated with goat anti-mouse IgG alkaline phosphatase conjugate (Invitrogen, USA) diluted 1:5000 in T-PBS for 1 h at room temperature. After rinsing in T-PBS, the membrane was developed with NBT-BCIP substrate system (Invitrogen, USA) for 10 min in the dark. The reaction was stopped by distilled water and the membrane was air dried.

Results and Discussion

Lymphocystis disease occurs in approximately 100 marine and freshwater fish species (Wolf, 1988) and is characterized by clusters of enlarged hypertrophied dermal cells on the skin and fins of affected fish (Wolf, 1998). In the present study, LCDVs were detected and purified from the marine fish (olive flounder and rockfish) and the freshwater ornamental fish (gourami and painted glassfish). The affected fish had cluster of tumors or nodules on the skin, fins and oral lips, composed of hypertrophied dermal cells suggesting that they are related to *Lymphocystis* genus (Walker, 1962; Samalecos, 1986; Anderson *et al*., 1993).

LCDV was confirmed by PCR amplification using specific primers sets for the MCP gene (Table. 1, Fig. 1). Approximately 1.3 kbp of PCR product was obtained from olive flounder isolate with the LCC-F&R primer set, but not with LCDVs F&R primer set. Approximately 600 bp of PCR product was obtained from painted glass fish, gourami and rockfish isolates with only the LCDVs F&R primer set. These results suggest that there is variation in nucleotide sequences between virus detected from olive flounder and viruses detected from painted glass fish, gourami and rockfish.

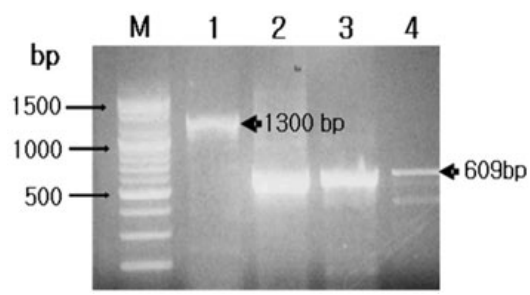

Fig. 1. PCR detection of MCP genes of different fish LCDV isolates (arrows). M: molecular marker, 1: olive flounder isolate, 2: painted glass fish isolate, 3: gourami isolate, and 4: rockfish isolate.

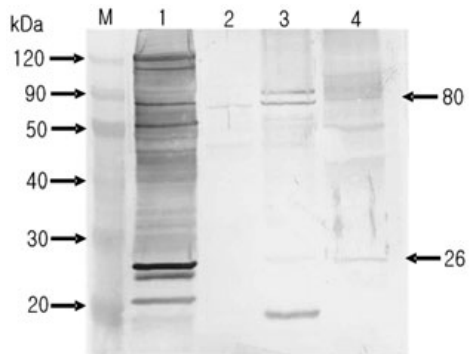

Fig. 2. Western blot analysis of LCDVs from marine and freshwater fish (arrows showing common antigenic bands). M: molecular marker, 1: olive flounder isolate, 2: painted glass fish isolate, 3: gourami isolate and 4: rockfish isolate.

In western blotting analysis using anti-LCDV mouse polyclonal serum, major antigenic proteins had 21, 26, 45, 50, 80, 110 and 120 kDa in olive flounder, 26, 47 and 80 kDa in painted glass fish, 26, 46, 80 and 92 kDa in gourami, 26, 44, 49, 80 and 105 in rockfish, respectively (Fig. 2). All the marine and freshwater isolates showed only common antigens of approximately 26 kDa and 80 kDa, although all the isolates showed a common band of approximately 50 kDa in SDS-PAGE, which is presumed size of MCP of LCDV (data not shown). Iwamoto *et al*. (2002) reported different antigenic protein profiles among LCDV isolates collected from Japanese flounder, with common protein of 50 kDa, which was different with our results obtained from LCDV proteins of other fish species. These results suggest that antigenic protein profiles of LCDVs may vary depending upon fish species.

Acknowledgements

This research was partially supported by the MKE (The Ministry of Knowledge Economy), Korea, under an ITRC (Information Technology Research Center) support program supervised by the IITA (Institute for Information Technology Advancement) (IITA-2009-C1090-0902-0001). This work was conducted as a part of the first author's Doctoral research program in Korea (Financially supported by Grants in KRF-2006-11-F00015).

References

Anderson, I.G., Prior, H.C., Rodwell, B.J. and Harris, G.O.: Iridovirus-like virions in imported dwarf gourami (*Colisa lalia*) with systemic amoebiasis. Aust. Vet. J. 70:66-67, 1993.

Cano, I., Alonso, M.C., Garcia-Rosado, E., Rodrigues Saint-Jean, S., Castro, D. and Borrego, J.J.: Detection of lymphocystis disease virus (LCDV) in asymptomatic cultured gilt-head sea bream (*Sparus aurata*, L.) using an immunoblot technique. Vet. Microbiol. 113:137-141, 2006.

Darai, G., Anders, K., Koch, H.G., Delius, H., Gelderblom, H., Samalecos, C. and Flügel, R.M.: Analysis of the genome of fish lymphocystis disease virus isolated directly from epidermal tumours of pleuronectes. Virology 126:466-479, 1983.

Flugel, R.M., Darai, G. and Gelderblom, H.: Viral proteins and adenosine triphosphate phosphohydrolase activity of fish lymphocystis disease virus. Virology 122:48-55, 1982.

Garcia-Rosado, E., Castro, D., Cano, I., Alonso, C.M., Perez-Prieto, I.S. and Borrego, J.J.: Protein and glycoprotein content of lymphocystis disease virus (LCDV). Int. Microb. 7:121-126, 2004.

Iwamoto, R., Hasegawa, O., LaPatra, S. and Yosumizu, M.: Isolation and characterization of the Japanese flounder (*Paralichthys olivaceus*) lymphocystis disease virus. J. Aquat. Animal Health 14:114-123, 2002.

Kitamura, S.I., Jung, S.J., Kim, W.S., Nishizawa, T., Yoshimizu, M. and Oh, M.J.: A new genotype of lymphocystivirus, LCDV-RK, from lymphocystis diseased rockfish. Arch. Virol. 151:607-615, 2006a.

Kitamura, S.I., Jung, S.J. and Oh, M.J.: Differentiation of lymphocystis disease virus genotype by multiplex PCR. J. Microbiol. 44:248-253, 2006b.

Laemmli, U.K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685, 1970.

Samalecos, C.P.: Analysis of the structure of fish lymphocystis disease virions from skin tumors of *Pleuronectes*. Arch. Virol. 91:1-10, 1986.

Schnitzler, P. and Darai, G.: Identification of the gene encoding the major capsid protein from fish lymphocystis disease virus. J. Gen. Virol. 74:2143-2150, 1993.

Tidona, C.A. and Darai, G.: The complete DNA sequence of lymphocystis disease virus. Virology 230:353-362, 1997.

Tidona, C.A., Schnitzler, P., Kehm, R. and Darai, G.: "Is the major capsid protein of iridoviruses a suitable target for the study of viral evolution?" Virus Genes 16:59-66, 1998.

Walker, R.: Fine structure of lymphocystis disease virus of fish. Virology 18:503-505, 1962.

Webby, R. and Kalmakoff, J.: Sequence comparison of the major capsid protein gene from 18 diverse iridoviruses. Arch. Virol. 143:1949-1966, 1998.

Wolf, K.: Lymphocystis disease. In: Fish viruses and fish viral diseases (ed. By K. Wolf). Cornell University Press, Ithaca, p., 268-291, 1988.

Zhang, Q.Y., Xiao, F., Xie, J., Li, Z.Q. and Gui, J.F.: Complete genome sequence of lymphocystis disease virus isolated from China. J. Virol. 78:6982-6994, 2004.

Zwillenberg, L.D. and Wolf, K.: Ultrastructure of lymphocystis virus. J. Virol. 2:393-399, 1968.

Manuscript Received : February 23, 2009
Revised : July 1, 2009
Accepted : July 15, 2009