PF-2

Differentiation of lymphocystis disease virus genotype by multiplex PCR

Jin-Yong Ko, Shin-Ichi Kitamura, Sung-Ju Jung, Myung-Joo Oh*
Department of Aqualife Medicine, Chonnam National University, Yeosu 550-749, Korea

Introduction

Lymphocystis disease virus (LCDV), the causative agent of lymphocystis disease (LCD), belongs to the genus *Lymphocystivirus* within the family *Iridoviridae*. In Korea, LCD is a common fish disease, mainly affects flounder, *Paralichthys olivaceus*, rockfish *Sebastes schlegeli* and sea bass, *Lateolabrax* sp. The major capsid protein (MCP) gene of the viruses is the most important with regard to analysis of genetic relationships among iridoviruses. Recently, we reported that at least three genotypes are present in the genus, based on MCP gene sequences and the pathogenicities of the viruses; genotype I includes LCDV-1, genotype II consists of flounder isolates and the genotype III consists of rockfish isolates (Kitamura *et al.*, 2006). To date, little is known about molecular detection method for the virus. In this study, a multiplex PCR based on MCP gene was developed, to enable the fast detection and typing of the viruses. We also analyzed the MCP gene of a new isolate from sea bass.

Materials and Methods

Ten LCDV isolates from flounder and rockfish, and the one new isolate from sea bass, SB98Yosu, were used. In order to clarify the genetic position of the SB98Yosu, sequencing of the MCP gene was performed. For the multiplex PCR, the primer sets were designed based on MCP gene sequence information. Three hyper-variable regions were selected for the design of forward primers (GI, GII and GIII), and one reverse primer (LCDVs-R) was designed on a conserved region. Also, one universal forward primer (LCDVs-F), which was designed to detect all of these viral genotypes, was based on the conserved region. The primer sequences are were as follows: 5′-YTGGTTCAGTAAATTACCRG-3′ - LCDVs-F ; 5′-GTAATCCATACTTGHACRTC-3′ - LCDVs-R ; 5′-TTAGATTATTGGGCAGCGTT-3′ - GI; 5′-TYGATTCCAAYGGTCAATTA-3′ - GII; 5′-AGGAAATAACAACCGTATGAATGCA-3′ -GIII. Initially, the specificity of

each primer set was tested. Secondly, for the multiplex PCR, 20 M of each primer (GI, GII and GIII forward primers and LCDVs-R reverse primer) was mixed and used for the reactions. All PCR amplifications were performed for 30 cycles (95℃ for 1 min, 5 5℃ for 1 min, and 72℃ for 1 min).

Results and Discusion

Nucleotide sequence analysis revealed that the PCR product of the SB98Yosu isolate was 1356 bases in length. A phylogenetic tree based on the MCP gene nucleotide sequences revealed that the SB98Yosu isolate belongs to the genotype II along with the flounder isolates.

PCR product of 609 bases was obtained from three isolates including JF03GunNeA (genotype II), SB98Yosu (genotype II) and RF03Yosu (genotype III) using the LCDVs-F and LCDVs-R universal primer set. Also, the PCR reaction gave rise to highly specific PCR products for the individual isolates, including JF03GunNeA (250 bases), SB98Yosu (250 bases) and RF03Yosu (468 bases), using GII or GIII forward primers and LCDVs-R primer. Thus, the results strongly suggest that the primer sets can, at least, distinguish clearly between LCDV isolates from genotypes II and III. To evaluate the practical application of the multiplex PCR, two or three LCDV genomes (JF03GunNeA, SB98Yosu and RF03Yosu) were mixed. Amplicons corresponding to each isolate were generated from the mixed DNA specimens. Thus, the multiplex PCR had a capacity to distinguish at least two genotypes of viruses, even in a sample including more than 2 viral genotypes. We present have developed a method for the detection and differentiation of LCDV isolates, which can be applied in all any laboratory containing standard PCR equipment.

References

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