J. Fish. Sci. Technol. 8(3), 185-187, 2005

JOURNAL OF
FISHERIES SCIENCE
AND TECHNOLOGY

Notes

Detection of Fish Rhabdoviruses using a Diagnostic Fish Rhabdovirus DNA Chip

Young Ju Kim and Myung Suk Lee*

Department of Microbiology, Pukyong National University, Busan 608-737, Korea

We tested the in vivo ability of a DNA chip to detect virus-specific genes from virus-infected olive flounder *Paralichthys olivaceus* and rainbow trout *Oncorhynchus mykiss*. Target cDNA was obtained from total RNA of virus infected cell lines by reverse transcription (RT) and was labeled with fluorescent dye (Cy5-dUTP). The results show the successful detection of infectious hematopoietic necrosis virus (IHNV) and viral hemorrhagic septicaemia virus (VHSV) genes in the virus-infected fishes.

Key words: Rhabdovirus, In vivo detection, DNA chip, *Paralichthys olivaceus*, *Oncorhynchus mykiss*

Introduction

Several techniques of molecular biology, such as enzyme linked immunosorbent assay (ELISA; Dixon and Hill, 1984), Western blotting (Rimstad et al., 1990), and fluorescent antibody testing (Hsu et al., 1985), have been used to diagnose virus infection in diseased fishes. The reverse transcriptase-polymerase chain reaction (RT-PCR) has also been employed in the detection of viral RNA (Koutna et al., 2003). However, these methods are difficult to apply in the early stages of infection when the virus titer is low (Kim, 1999). Moreover, RT-PCR requires highly purified RNA (Koutna et al., 2003). It is important to rapidly identify and differentiate the causative virus in infected fish, because rapid determination of the origin of an outbreak may help to prevent the further spread of the disease (Willams et al., 1999).

At present, the best method of viral disease control is avoidance of virus introduction through rapid detection (Vernet, 2002). In this study, we applied a DNA chip to detect and identify rhabdoviruses in infected fishes olive flounder *Paralichthys olivaceus* and rainbow trout *Oncorhynchus mykiss*.

*Corresponding author: leems@pknu.ac.kr

Materials and Methods

Target cDNA preparation and hybridization

A-ll experimental procedures were carried out as described in Kim et al. (2005).

Preparation of target cDNA from diseased olive flounder and rainbow trout

To evaluate the efficiency of the DNA chip for virus detection in vivo, virus-infected flounder and rainbow trout samples were collected. Flounder were collected in Gangneung (Korea) and trout were collected in Sangju (Korea). Total RNA was isolated from brain, liver, and kidney tissues of virus-infected fish using TRIzol reagent (Invitrogen, USA). Tissues were homogenized with 3 ml of TRIzol reagent, and the RNA was extracted by the mentioned method in a previously thesis (2005-45) (Kim, 2003). Total RNA (60-150 μg) was labeled with oligo$(dT)_{18}$ in a reverse transcription reaction. Cy5-dUTP (Amersham Biosciences, UK) was used as a fluorescent label for the cDNA, and the subsequent procedures were performed as described previously (Bowtell and Sambrook, 2003; Tran et al., 2002).

Hybridization and scanning

Hybridization, washing, and scanning were also conducted as in the previous study (Schena, 2003).

Results and Discussion

Two virus-infected flounder and rainbow trout samples were used to evaluate the efficiency of the DNA chip for virus detection in vivo. Total RNA was extracted from samples, and cDNA was synthesized from both total RNAs and hybridized with a prepared DNA chip. Fig. 1 shows the virus detection results in olive flounder. Total RNA concentrations were 60 μg (A), 100 μg (B), and 150 μg (C). The signal spots indicated only viral hemorrhagic septicaemia virus (VHSV), not infectious hematopoietic necrosis virus (IHNV) or hirame rhabdovirus (HIRRV), although the intensity differed according to the RNA concentration. This result lead to the conclusion that this fish sample was infected only by VHSV.

Fig. 2 shows the virus detection result for the rainbow trout. Total RNA concentration was adjusted to 70 μg. The signal spots indicated only IHNV, although the intensity was stronger in the N and M1 gene, but lower in the G gene. No signal spots appeared indicating VHSV and HIRRV. Consequently, this sample was infected by IHNV.

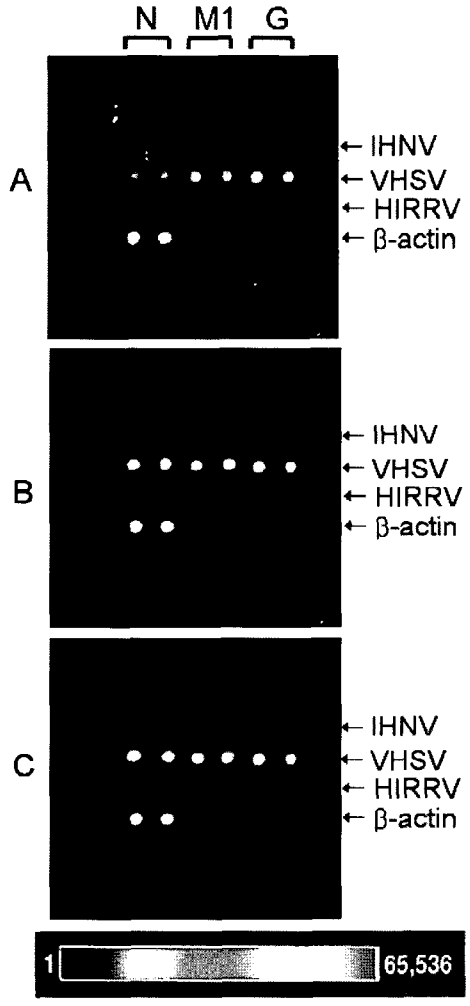

Fig. 1. Detection of VHSV in infected olive flounder using DNA chip. A, 60 μg of total RNA, B, 100 μg of total RNA; C, 150 μg of total RNA. Color bar represents the intensity of signal.

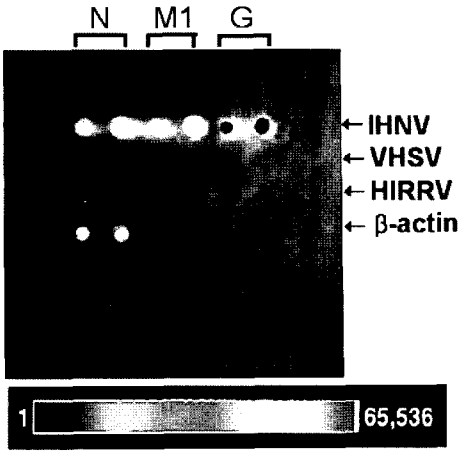

Fig. 2. Detection of IHNV in infected rainbow trout using DNA chip. Total RNA concentration was 70 μg. The color bar represents the intensity of signal.

In conclusion, we detected using a DNA chip viral rhabdoviruses, including IHNV, VHSV, and HIRRV. We expect that such a DNA chip preparation will help to detect other viruses in various fishes.

Acknowledgments

This work was supported by grant No. 20010064 of the Korean Ministry of Maritime Affairs and Fisheries.

References

Bowtell, D. and J. Sambrook. 2003. DNA Microarrays, Cold Spring Habor Laboratory Press, New York, pp. 79-256.

Dixon, P.F. and B. J. Hill. 1984. Rapid detection of fish rhabdoviruses by the enzyme-linked immunosorbent assay (ELISA). Aquaculture, 42, 1-12.

Hus, Y.L. and J.C. Leong. 1985. A comparison of detection methods for infectious hematopoietic necrosis virus. J. Fish Dis., 8, 1-12.

Kim, S.J. 1999. Comparison of infectious hemato-poietic necrosis virus detection methods. MS thesis, Pukyong National University, Korea, pp. 1-36.

Kim, S.M. 2003. Infection of viral hemorrhagic septicemia virus (VHSV) in cultured olive flounder, *Paralichtyys olivaceus*. Ph.D. thesis, Pukyong National University, Korea, pp. 1-21.

Kim, Y.J., J.H. Kang, S.M. Kim, S.I. Park, S.B. Kim and M.S. Lee. 2005. Optimization of a diagnostic DNA chip for fish rhabdovirus. J. Fish. Sci. Technol., 8, 122-127.

Koutna, M., T. Vesely, I. Psikal and J. hulova. 2003. Identification of spring viraemia of carp virus (SVCV) by combined RT-PCR and nested PCR. Dis. Aquat. Org., 55, 229-235.

Rimstad, E., R. Krona, E. Hornes, Q. Olsvik and B. Hyllseth. 1990. Detection of infectious pancreas-tic necrosis virus (IPNV) RNA by hybridization with an oligonucleotide DNA probe. Vet. Microbiol., 23, 211-219.

Schena, M. 2003. Microarray Analysis. Wiley-Liss Book Co., New Jersey, pp. 1-25.

Tran, P.H., D.A. Peiffer, Y. Shin, L.M. Meek, J.P. Brody and K.W. Cho. 2002. Microarray optimization:increasing spot accuracy and automated identification of true microarray signals. Nucl. Acids Res., e54.

Vernet, G.. 2002. DNA chip technology and infectious diseases. Virus Res., 82, 65-71.

Willams, K., S. Blake, A. Sweeney, J.T. Singerand and B.L. Nicholson. 1999. Multiplex reverse transcriptase PCR assay for simultaneous detec-tion of three fish viruses. J. Clin. Microbiol., 37, 4139-4141.

(Received May 2005, Accepted September 2005)