Detection and Molecular Characterization of Enteroviruses in Korean Surface Water by Using Integrated Cell Culture Multiplex RT-PCR

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Objective To identify waterborne enteric viruses in Korean surface water. **Methods** Integrated cell culture(ICC)multiplex reverse transcription-polymerase chain reaction (RT-PCR) was simultaneously designed to detect coxsackieviruses (CV), polioviruses (PV), and reoviruses (RV). ICC-multiplex RT-PCR and phylogenetic analysis were conducted using 21 total culturable virus assay (TCVA)-positive sample-inoculated cell cultures. **Results** CV and RV were detected in 9 samples each, and 3 samples were positive for both CV and RV. PV was not detected in any sample. Molecular phylogenetic analysis of the VP1 gene sequences revealed that CV types B2 and B4 predominated in Korean surface water, and the nucleotide sequences of CV type B2 were clustered with those of CVs isolated from China and Japan. The results suggested that the evolution of these viruses occurred in a region-specific manner. **Conclusion** CV and RV are detectable in Korean surface water, with a predominance of CV type B2, and the evolution of CV type B2 occur in a region-specific manner.

Key words: ICC-multiplex RT-PCR; Coxsackieviruses; Molecular phylogeny

REFERENCES

- 1. Abbaszadegan M, Huber M S, Gerba C P, *et al.* (1993). Detection of enteroviruses in groundwater with the polymerase chain reaction. *Appl Environ Microbiol* **59**(5), 1318-1324.
- Fout G S, Martinson B C, Moyer M W, et al. (2003). A multiplex reverse transcription-PCR method for detection of human enteric viruses in groundwater. *Appl Environ Microbiol* 69(6), 3158-3164.
- Lee G C, Jee Y S, Lee C H, *et al.* (2006). Influence of physicochemical environmental factors on the occurrence of waterborne viruses in Korean surface water. *J Bacteriol Virol* 36(4), 1-7. (In Korean)
- Muscillo M, Carducci A, La Rosa G, *et al.* (1997). Enteric virus detection in Adriatic seawater by cell culture, polymerase chain reaction, and polyacrylamide gel electrophoresis. *Water Res* 31(8), 1980-1984.
- 5. Papaventsis D, Siafakas N, Markoulatos P, *et al.* (2005). Membrane adsorption with direct cell culture combined with reverse transcription-PCR as a fast method for identifying enteroviruses from sewage. *Appl Environ Microbiol* **71**(1), 72-79.
- Beller M, Ellis A, Lee S H, *et al.* (1997). Outbreak of viral gastroenteritis due to a contaminated well: international consequences. *JAMA* 278(7), 563-568.
- Norder H, Bjerregaard L, Magnius L, et al. (2003). Sequencing of 'untypable' enteroviruses reveals two new types, EV-77 and EV-78, within human enterovirus type B and substitutions in the BC loop of the VP1 protein for known types. J Gen Virol

84(4), 827-836.

- Oberste M, Schnurr D, Maher K, et al. (2001). Molecular identification of new picornaviruses and characterization of a proposed enterovirus 73 serotype. J Gen Virol 82(2), 409-416.
- 9. Thapar N, Sanderson I R (2004). Diarrhoea in children: An interface between developing and developed countries. *Lancet* **363**(9409), 641-653.
- Ahn J, Choi J, Joo C H, et al. (2004). Susceptibility of mouse primary cortical neuronal cells to coxsackievirus B. J Gen Virol 85(6), 1555-1564.
- 11.Modlin J F (1995). Coxsackieviruses, echoviruses and newer enteroviruses. In *Principles and practice of infectious diseases*. 4th ed. (G. L. Mandell, *et al.* Eds.), pp. 1620-1636. Churchill Livingstone, New York.
- 12. Yang C F, Chen H Y, Jorba J, *et al.* (2005). Intratypic recombination among lineages of type 1 vaccine-derived poliovirus emerging during chronic infection of an immunodeficient patient. *J Virol* **79**(20), 12623-12634.
- 13. Hyypiä T, Stanway G (1993). Biology of coxsackie a viruses. *Adv Virus Res* **42**, 343-373.
- 14. Tyler K L, Sokol R J, Oberhaus S M, et al. (1998). Detection of reovirus RNA in hepatobiliary tissues from patients with extrahepatic biliary atresia and choledochal cysts. *Hepatology* 27(6), 1475-1482.
- Ko G, Cromeans T L, Sobsey M D (2003). Detection of infectious adenovirus in cell culture by mRNA reverse transcription-PCR. *Appl Environ Microbiol* 69(12), 7377-7384.
- 16.U. S. Environmental Protection Agency (1996). ICR microbial laboratory manual. Publication EPA/600/R-95/178. Office of Research and Development, Government Printing Office, Washington, D.C.

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- 17. Oberste M S, Nix W A, Maher K, *et al.* (2003). Improved molecular identification of enteroviruses by RT PCR and amplicon sequencing. *J Clin Virol* **26**(3), 375-377.
- 18. Thompson J D, Gibson T J, Plewniak F, et al. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 25(24), 4876-4882.
- Felsenstein J (1993). PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle.
- 20.Matsushita T, Matsui Y, Shirasaki N (2006). Analysing mass balance of viruses in a coagulation-ceramic microfiltration hybrid system by a combination of the polymerase chain reaction (PCR) method and the plaque forming units (PFU) method. *Water Sci Tech* 53(7), 199-207.
- 21.Rajal V B, McSwain B S, Thompson D E, *et al.* (2007). Validation of hollow fiber ultrafiltration and real-time PCR using bacteriophage PP7 as surrogate for the quantification of viruses from water samples. *Water Res* **41**(7), 1411-1422.
- Reynolds K A (2004). Integrated cell culture/PCR for detection of enteric viruses in environmental samples. *Methods Mol Biol* 268, 69-78.
- 23.Spinner M L, Di Giovanni G D (2001). Detection and identification of mammalian reoviruses in surface water by combined cell culture and reverse transcription-PCR. *Appl Environ Microbiol* 67(7), 3016-3020.
- 24.Huang Y T, Yam P, Yan H, et al. (2002). Engineered BGMK cells for sensitive and rapid detection of enteroviruses. J Clin Microbiol 40(2), 366-371.
- 25.Bolanaki E, Kottaridi C, Markoulatos P, et al. (2005). Nucleotide analysis and phylogenetic study of the homology

boundaries of caxsackie A and B viruses. Virus Genes 31(3), 307-320.

- 26.Caro V, Guillot S, Delpeyroux F, et al. (2001). Molecular strategy for 'serotyping' of human enteroviruses. J Gen Virol 82(1), 79-91.
- 27.Oberste M S, Maher K, Kilpatrick D R, et al. (1999). Typing of human enteroviruses by partial sequencing of VP1. J Clin Microbiol 37(5), 1288-1293.
- 28. Thoelen I, Moes E, Lemey P, et al. (2004). Analysis of the serotype and genotype correlation of VP1 and the 5' noncoding region in an epidemiological survey of the human enterovirus B species. J Clin Microbiol 42(3), 963-971.
- 29. Cheon D S, Lee J, Lee K, et al. (2004). Isolation and molecular identification of echovirus 13 isolated from patients of aseptic meningitis in Korea, 2002. J Med Virol 73(3), 439-442.
- 30.Li L, Shimizu H, Doan L T, *et al.* (2004). Characterizations of adenovirus type 41 isolates from children with acute gastroenteritis in Japan, Vietnam, and Korea. *J Clin Microbiol* 42(9), 4032-4039.
- 31.Park C S, Kim M S, Lee S D, et al. (2006). Molecular phylogenetic analysis of HIV-1 vif gene from Korean isolates. J Microbiol 44(6), 655-659.
- 32.Cloete T E, Da Silva E, Nel L H (1998). Removal of waterborne human enteric viruses and coliphages with oxidized coal. *Curr Microbiol* **37**(1), 23-27.
- 33.Iwai M, Yoshida H, Matsuura K, et al. (2006). Molecular epidemiology of echoviruses 11 and 13, based on an environmental surveillance conducted in Toyama Prefecture, 2002-2003. Appl Environ Microbiol 72(9), 6381-6387.

(Received November 21, 2007 Accepted July 5, 2008)