RESEARCH REPORT - No. 1203 FY 2012–2013

Title of research project	Development and application of genetic methods to detect infectious viruses for risk analysis of foodborne viruses in foods
Research project no.	(1203)
Research period	FY 2012–2013
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【Abstract】

Viral genome detection methods are exclusively used to detect foodborne viruses such as norovirus, hepatitis A and E viruses because it is difficult or impossible for such viruses to propagate in cell culture. For risk analysis of foodborne viruses in foods, a great disadvantage of genetic methods is that viral RNAs derived from non-infectious (non-intact) viral particles are detected. For the selective detection of viruses in food and other samples, we have developed a genetic method followed by quantitative detection using real-time PCR, in which both RNase ONE^{TM} ribonuclease digestion before RNA extraction and reverse transcription using oligo-d(T) primers are employed.

To evaluate the usefulness of the newly developed method, we compared RNA copy numbers measured by both the developed and conventional methods with titers of infectious viral particles using norovirus and feline calicivirus after heating, ultraviolet light irradiation, or sodium hypochlorite treatment. RNA copy numbers measured by the developed method correlated more closely with titers of infectious particles than those measured by the conventional method. The same results were obtained in bench-scale survival experiments under dried or aquatic conditions with or without organic substances and in field survival experiments in marine water in summer or winter. These survival experiments also showed that norovirus survived longer in the condition with organic substances than in the condition without organic substances and that it survived longer in winter than in summer.

We applied the developed method to detect norovirus contaminated naturally in waste water and oysters. The results showed that non-infectious norovirus particles existed at a higher rate in effluent waste water than in influent waste water and were accumulated in oysters, suggesting that some of the norovirus particles had been inactivated during treatments in waste water plants and then accumulated in oysters with further inactivation by physical mechanisms such as atmospheric temperature, ultraviolet light in sunlight, or other agents when they were floating in a river or sea water. Our experiments also showed that there is a difference between the stability of norovirus and that of feline calicivirus, especially in effluent waste water or marine water.

On the other hand, it is necessary to develop a simple method to recover antibody-covered viral particles and antibody-non-covered viral particles separately because the former may not be infectious. Therefore, we have also developed two methods to recover them separately using a protein A column or PANSORBIN® cells with or without anti-human IgA antibody. Antibody-covered norovirus particles and antibody-non-covered norovirus particles in stool samples were recovered separately by both methods. Protein A column treatment

experiments also showed that the percentage of IgG-covered viral particles in total viral particles in stool samples was higher in asymptomatic patients (17.9%) than in symptomatic patients (14.5%).

Heat inactivation experiments for hepatitis A and E viruses were carried out to obtain data for the conditions of their inactivation. An antigen-detection ELISA for hepatitis E virus has been developed.

The results suggest that our developed methods are useful to detect infectious viruses in food, waste water, sea water, and environmental swab or clinical samples, and the data obtained by using the developed methods are helpful for risk analysis of foodborne viruses in foods.