JOURNAL OF
INTEGRATED FIELD SCIENCE
Field Science Center, Graduate School of Agricultural Science, Tohoku University

Symposium mini review

Detection and Epidemiological Analysis of Symbiotic Viruses from Protozoa

Fumi MURAKOSHI[1,2], Takaaki NAKAYA[1] and Kentaro KATO[2]

[1]Graduate School of Medical Science, Kyoto Prefectural University of Medicine
[2]Graduate School of Agricultural Science, Tohoku University

Keywords
Cryptosporidium, protozoan virus, Cryptosporidium parvum virus 1, CSpV1

Corresponding Author
Fumi MURAKOSHI,
muraf@koto.kpu-m.ac.jp

Abstract

Various symbiotic viruses exist in protozoan parasite. Most species classified with the family *Totiviridae* or *Partitiviridae*, and are transmitted from cell to cell during cell division. Some symbiotic viruses have been reported to influence the pathogenicity of symbiotic parasites to their hosts. There are also reports of the use of symbiotic viruses in the epidemiological analysis of protozoan parasites. We have demonstrated that Cryptosporidium parvum virus 1 (CSpV1), a symbiotic virus of *Cryptosporidium parvum*, can be a high-resolution tool to trace *C. parvum*.

Introduction

The existence of symbiotic viruses in protozoan parasite has been suggested by electron microscopic observation. To date, protozoan viruses have been reported from various protozoa. Common features of these are having double-stranded RNA (dsRNA) and transmitting from cell to cell during cell division. *Cryptosporidium* virus belongs to family *Partitiviridae*, whereas other protozoal symbiotic viruses are classified as family *Totiviridae*. Symbiotic viruses have also been reported from plants and bacteria, and there are reports of some effects on the host of the symbiotic virus. For example, a bacteriophage symbiotic with *Pseudomonas aeruginosa* inhibits the clearance of *P. aeruginosa* from infected wounds (Sweere *et al*., 2019).

Recently, it has been reported that symbiotic viruses of *Leishmania* and *Trichomonas* affect pathogenicity and expression of surface antigens (Ives *et al*., 2011; Wang *et al*., 1987). However, the effects of other protozoan symbiotic viruses on its hosts are often unknown. Moreover, regarding protozoan symbiosis viruses, there are few reports on detection and epidemiological studies. Therefore, we conducted an epidemiological analysis of symbiotic viruses of protozoa, and also investigated the effects of symbiotic viruses on the host.

Detection of protozoal symbiotic viruses

Protozoan symbiotic viruses can be identified by electron microscopy. Since all protozoan symbiotic viruses detected at present are dsRNA viruses, dsRNA can be detected by extracting all nucleic acids from parasites, treating them with DNase and RNase, and performing electrophoresis. This is because dsRNA is resistant to DNase and RNase treatment. Alternatively, the entire nucleic acid is adsorbed on a dsRNA-specific column and electrophoresed (**Fig. 1**). However, because these methods require a large number of protozoa, they are often analyzed using next-generation sequencers. Symbiotic viruses with known sequences can be detected by PCR using primers. It is also possible to detect symbiotic viruses using dsRNA antibodies (Zangger *et al*., 2013).

Symbiotic viruses of *Cryptosporidium*

Cryptosporidium infects various animals and causes severe diarrhea. Because of the absence of effective drugs, infection in immunocompromised patients is fatal. Recently, dsRNA

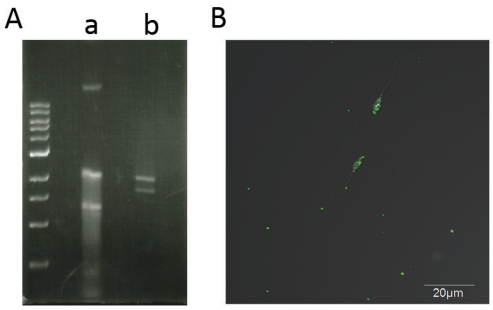

Fig. 1. Detection of dsRNA from protozoa. (A) Electrophoresis of dsRNA extracted from *Cryptosporidium parvum*. a: total nucleic acid, b: purified dsRNA. (B) Immunofluorescence assay (IFA) of *Leishmania major* using dsRNA antibody.

symbiotic virus belonging to the family Partitiviridae has also been reported in these protozoa and has been classified as *Cryspovirus* (Nibert *et al*., 2009, 2017). This virus contains two unrelated, liner dsRNA segments of 1.7 kbp (dsRNA1) and 1.4 kbp (dsRNA2) that are encapsulated separately. dsRNA 1 is RNA dependent RNA polymerase (RdRp) and dsRNA2 encodes the capsid protein (CP). This symbiotic virus has also been detected in *C. hominis*, *C. felis* and *C. meleagridis* (Leoni *et al*., 2003. 2006). *Cryptosporidium* symbiotic viruses are thought to exist as approximately 2,000 particles in an oocyst (Kniel *et al*., 2004). Therefore, studies have been conducted to detect *C. parvum* with high sensitivity using the *Cryspovirus* antibody (Tai *et al*., 2019).

The 60-kDa glycoprotein (GP60) gene is the major subtyping gene of *Cryptosporidium*. The most major subtype of *C. parvum* is the IIa subtype, which is further classified by the number of trinucleotide repeats encoding serine. However, in Japan, the GP60 subtype of *C. parvum* detected in cattle is mostly the IIaA15G2R1 subtype. Therefore, there is a problem that the subtyping by the GP 60 gene cannot be used in the investigation of the infection source. Therefore, we tested whether Cryptosporidium parvum virus 1 (CSpV1) could be used to trace *C. parvum* infection sites. Because RNA viruses show higher mutation frequencies than their hosts because they lack proofreading enzymes.

As a result, the sequence of CSpV1 detected from *C. parvum* IIaA15G2R1 collected throughout Japan reflected the infection site by phylogenetic analysis. Thus, it became clear that CSpV1 could be used as a tool for infection-site estimation and host tracking (Murakoshi *et al*., 2016).

Symbiotic viruses of *Eimeria*

Eimeria spp. infects various animals and causes diarrhea. The *Eimeria* symbiotic viruses of which sequences are reported are E. tenella RNA virus 1, E. brunetti RNA virus 1, E. necatrix RNA virus 1 and E. stiedai RNA virus 1 (Wu *et al*., 2016; unpublished; Lee and Fernando, 1998; Reets *et al*., 1989), which have been detected in each chicken *Eimeria* species. *Totiviridae* is a single-stranded dsRNA virus coding RNA dependent RNA polymerase (RdRp) and the capsid protein (CP). These are all single reports and the effect on the prevalence and pathogenicity of *Eimeria* symbiotic viruses are unknown.

We are currently conducting an epidemiological analysis of the symbiotic virus in chicken *Eimeria* in Japan, and suggested that the symbiotic virus exists at a high frequency (data not shown).

Symbiotic viruses of *Leishmania*, *Giardia*, and *Trichomonas*

Several other protozoan parasites including *Leishmania*, *Trichomonas*, and *Giardia* are also known to have symbiotic dsRNA viruses (family *Totiviridae*): Leishmania RNA viruses (LRVs) 1 and 2; Trichomonas vaginalis viruses (TVVs) 1, 2, 3, and 4; and Giardia lambia virus (GLV).

In *Trichomonas*, the presence of dsRNA has been reported to be involved in the expression of surface antigens of *Trichomonas* (Wang *et al*., 1987). In *Leishmania*, *L. guyanensis* in which LRV1 is present was reported to have significantly increased lesion size in mice (Ives *et al*., 2011).

We are currently investigating whether a similar phenomenon occurs in the presence of symbiotic viruses in *L. major*, we have obtained data that LRV2 is also involved in pathogenicity (data not shown).

Acknowledgments

I would like to thank the Organizing Committee of the 17th International Symposium on Integrated Field Science for the invitation to participate at this event. This work was supported by grants-in-aid for Young Scientists (B) and Scientific Research on Innovative Areas (3805).

References

Ives, A., C. Ronet, F. Prevel, G. Ruzzante, S. Fuertes-Marraco, F. Schutz, H. Zangger, M. Revaz-Breton, L. F. Lye, S. M. Hickerson, S. M. Beverley, H. Acha-Orbea, P. Launois, N. Fasel and S. masina (2011) Leishmania RNA virus controls the severity of mucocutaneous leishmaniasis. *Science*, 331: 775-778.

Kniel, K. E., J. A. Higgins, J. M. Trout, R. Fayer and M. C. Jenkins (2004) Characterization and potential use of a Cryptosporidium parvum virus (CPV) antigen for detecting *C. parvum* oocysts. *Journal of Microbiological Methods*, 58: 189-195.

Lee, S. and M. A. Fernando (1998) Intracellular localization of viral RNA in *Eimeria necatrix* of the domestic fowl. *Parasitology Research*, 84: 601-606.

Leoni, F., C. I. Gallimore, J. Green and J. McLauchlin (2003) Molecular epidemiological analysis of *Cryptosporidium* isolates from humans and animals by using a heteroduplex mobility assay and nucleic acid sequencing based on a small double-stranded RNA element. *Journal of Clinical Microbiology*, 41: 981-992.

Leoni, F., C. I. Gallimore, J. Green and J. McLauchlin (2006) Characterisation of small double stranded RNA molecule in *Cryptosporidium hominis*, *Cryptosporidium felis* and *Cryptosporidium meleagridis*. *Parasitology International*, 55: 299-306.

Murakoshi, F., M. Ichikawa-Seki, J. Aita, S. Yaita, A. Kinami, K. Fujimoto, Y. Nishikawa, S. Murakami, T. Horimoto and K. Kato (2016) Molecular epidemiological analyses of Cryptosporidium parvum virus 1 (CSpV1), a symbiotic virus of *Cryptosporidium parvum*, in Japan. *Virus Research*, 211: 69-72.

Nibert, M. L., K. M. Woods, S. J. Upton and S. A. Ghabrial (2009) *Cryspovirus*: a new genus of protozoan viruses in the family *Partitiviridae*. *Archives Virology*, 154: 1959-1965.

Revets, H., D. Dekegel, W. Deleersnijder, J. De Jonckheere, J. Peeters, E. Leysen and R. Hamers (1989) Identification of virus-like particles in *Eimeria stiedae*. *Molecular and Biochemical Parasitology*, 36: 209-215.

Sweere, J. M., J. D. Van Belleghem, H. Ishak, M. S. Bach, M. Popescu, V. Sunkari, G. Kaber, R. Manasherob, G. A. Suh, X. Cao, C. R. de vries, D. N. Lam, P. L. Marshall, M. Birukova, E. Katznelson, D. V. Lazzareschi, S. Balaji, S. G. Keswani, T. R. Hawn, P. R. Secor and P. L. Bollyky (2019) Bacteriophage trigger antiviral immunity and prevent clearance of bacterial infection. *Science*, 363.

Tai, L., J. Li, J. Yin, N. Zhang, J. Yang, H. Li, Z. Yang, P. Gong and X. Zhang (2019) A novel detection method of *Cryptosporidium parvum* infection in cattle based on Cryptosporidium parvum virus 1. *Acta Biochimica Biophysica Sinica (Shanghai)*, 51: 104-111.

Vong, M., J. G. Ludington, H. D. Ward and M. L. Nibert (2017) Complete cryspovirus genome sequences from *Cryptosporidium parvum* isolate Iowa. *Archives of Virology*, 162: 2875-2879.

Wang, A., C. C. Wang and J. F. Alderete (1987) *Trichomonas vaginalis* phenotypic variation occurs only among trichomonads infected with the double-stranded RNA virus. *Journal of Experimental Medicine*, 166: 142-150.

Wu, B., X. Zhang, P. Gong, M. Li, H. Ding, C. Xin, N. Zhao and J. Li

(2016) *Eimeria tenella*: a novel dsRNA virus in *E. tenella* and its complete genome sequence analysis. *Virus Genes*, 52: 244-252.

Zangger, H., C. Ronet, C. Desponds, F. M. Kuhlmann, J. Robinson, M. A. Hartley, F. Prevel, P. Castiglioni, F. Pratlong, P. Bastien, N. Müller, L. Parmentier, N. G. Saravia, S. M. Beverley and N. Fasel (2013) Detection of Leishmania RNA virus in *Leishmania* parasites. *PLoS Neglected Tropical Diseases*, 7: e2006.