

Ecology of viruses in rice fields

Makoto Kimura^A, Guanghua Wang^B, Natsuko Nakayama^C and Susumu Asakawa^D

^ANagoya University, Furocho, Chikusa, Nagoya, 464-8601 Japan, Email kimuram@agr.nagoya-u.ac.jp

^BNortheast Institute of Geography and Agroecology, CAS, Harbin, 150081, China, Email guanghuawang@hotmail.com

^CNagoya University, Furocho, Chikusa, Nagoya, 464-8601 Japan, Email natsun999@nifty.com

^DNagoya University, Furocho, Chikusa, Nagoya, 464-8601 Japan, Email asakawa@agr.nagoya-u.ac.jp

Abstract

Viruses are the most abundant biological entities in marine and freshwater environments. Many studies have shown the ecological importance of viruses as the greatest genomic reservoirs, in primary production and microbial food web in aquatic environments. However, the viral ecology in the soil environment has been little documented. In this study, we estimated viral abundance in the floodwater of rice fields, and then measured phage-infected bacterial cells for evaluating the importance of phage infection to bacterial hosts in the microbial food web. The diversity and specificity of *g23* sequences of T4-type bacteriophages in rice fields were also determined in comparison with those in aquatic environments. Viruses were also the most abundant biological entities in rice fields, and bacteriophages comprised the majority among viral communities. The high frequency of phage-infected bacterial cells indicated that the bacterial mortality from phage lysis could be significant enough to redirect the microbial food web and change the bacterial communities. The majority of *g23* sequences of T4-type bacteriophages in rice fields were distantly related to those of marine origins. The *g23* genes in rice fields have apparently diverged more compared to marine *g23* genes.

Key Words

Phage, *g23* gene, abundance, phage-infected bacterial cell, diversity, morphology

Introduction

Viruses are the most abundant biological entities in marine and freshwater environments. Many studies have indicated the ecological importance of viruses in global biogeochemical nutrient cycles. Viruses are recognised as the greatest genomic reservoirs in marine and freshwater environments due to their huge abundance and diversity. However, to date the ecology of soil viruses has been studied little at the community level.

We studied the seasonal variation in the abundance of viruses and bacteria in the floodwater of a rice field. Then, as phages comprised the majority of viral communities in the floodwater, the frequency of phage-infected bacterial cells in the floodwater was measured to evaluate the importance of phage communities in the microbial food web in rice fields. From the analysis of the capsid gene, *g23*, of the T4-type phages in rice field soils in Japan and Northeast China, many novel *g23* gene sequences were detected, which suggested the presence of soil-specific phage communities. These findings indicated the importance of viruses as genomic reservoirs and in the microbial food web in rice fields.

Materials and Methods

Enumeration of virus-like particles (VLPs) and their morphology

Enumeration of virus-like particles (VLPs) was performed by epifluorescence microscopy (EFM) after staining with SYBR Green I for the floodwaters collected from a Japanese rice field. Then the morphology of viruses was examined by transmission electron microscopy (TEM) for the same floodwater samples.

Frequency of visibly infected bacterial cells (FVIC)

The bacterial mortality in the floodwater due to phage lysis was evaluated by measuring the frequency of phage-infected bacterial cells with a transmission electron microscope (TEM). Floodwater samples were the same samples used for studying the viral abundance. The frequency of visibly infected bacterial cells (FVIC) was calculated from the proportion of phage-infected cells in the total number of cells examined.

Phylogeny and diversity of major capsid gene g23 in rice fields

To evaluate the phylogenetic characteristics and diversity of phages in rice field soils, *g23* genes of T4-type phages, *Myoviridae*, were PCR-amplified and sequenced from DNA extracted from rice field soils in Japan and Northeast China. *g23* genes in the floodwater were also studied by using the same floodwater samples mentioned above. The primers used for the *g23* amplification were MZIA1bis and MZIA6 (Filée *et al.* 2005). Denaturing gradient gel electrophoresis (DGGE) was conducted to the PCR products to efficiently obtain different *g23* clones.

Results and Discussion

Abundance and morphology of viruses in the floodwater

Viruses are the most abundant biological entities in the floodwater of rice fields. VLP abundance in the floodwater during rice cultivation period ranged from 5.6×10^6 to 1.2×10^9 VLPs/mL (mean abundance of 1.5×10^8 VLPs/mL). In contrast, VLP in the irrigation water ranged from 5.1×10^6 to 1.1×10^7 VLPs/mL. Smaller VLP abundance in the irrigation water than that in the floodwater indicated that viruses in the floodwater were not derived directly from the irrigation water but occurred in the floodwater. The high VLP abundance in the floodwater suggested that the floodwater is an aquatic environment abundant in viruses in comparison with marine, estuarine, and freshwater environments, where VLP abundance is generally less than 10^7 VLPs/mL (Wommack and Colwell 2000; Weinbauer 2004).

The virus-to-bacterium ratio (VBR) in the floodwater ranged from 0.11 to 72. VLP abundance showed significant correlation with bacterial abundance ($P < 0.01$). Although there was no significant correlation between VBR and VLP abundance, seasonal variation in VBR correlated significantly with the change of bacterial abundance, and VBR increased with the decrease in bacterial abundance ($P < 0.01$).

The capsid size distribution was monophasic, and the median values fell into the 50-60 nm or 60-70 nm fraction. More than half of the viruses fell within the size ranges from either 40-50 nm or 50-60 nm to either 60-70 nm or 70-80 nm for most samples. The predominance of viruses with the size classes of <100 nm was attributed to the predominance of phages in viral communities because the average capsid size for viruses of eukaryotic algae is reported to be 152 nm (Van Etten *et al.* 1991).

The most common capsid forms were tailed or non-tailed, isometric icosahedral form with a diameter of 50-70 nm, and other forms were too rare and sporadic to evaluate the difference in diversity among sampling dates from the morphology. Dominant viruses were estimated to be myoviruses (with a long contractile tail), siphoviruses (with a long non-contractile tail), and podoviruses (with a short non-contractile tail). Rough estimation of tailed viruses by TEM accounted for 2 to 54 % of the total in this study. As Ackermann (2001) summarised that 96% of phages were tailed among ca. 5,100 phages examined by TEM, the sonication used in our sample preparation might have caused tail loss in a considerable proportion of the viruses.

Frequency of phage-infected bacterial cells (FIC) in the floodwater

Weinbauer (2004) reviewed the virus-mediated carbon flow in pelagic oceans, where 6 to 26% of the carbon fixed by primary producers enters into the dissolved organic carbon (DOC) pool via virus-induced lysis at different trophic levels.

The FVIC ranged from 1.6 to 3.6 % for the floodwater samples. By TEM, phage particles are recognisable in the infected cells only in the late stage of the lytic cycle. Therefore, the frequency of phage-infected bacterial cells (FIC) was estimated from the FVIC by considering the fraction of the eclipse period in the latent period. The FIC ranged from 10.5% to 22.5 %, and the fraction of bacterial mortality from phage lysis was estimated to range from 12.8 to 35.0 % according to the non-linear steady-state model of Binder (1999). The frequency of phage lysis in floodwater was within the frequency ranges observed in other aquatic environments (Weinbauer 2004). The impact on bacterial mortality by phage lysis seemed to be large enough to redirect the microbial food web and induce the change and succession of bacterial communities in the floodwater since phages are, in general, strictly host-specific.

Characteristics and diversity of phages in rice field soils – estimation by g23 gene sequences of T4-type phages

Deduced amino acid residues of the partial *g23* fragments were aligned first together with the representatives of T-evens, PseudoT-evens, SchizoT-evens and ExoT-evens as well as marine *g23* clones. The majority of soil *g23* sequences were distantly related to the T-evens sequences and those of marine origin and formed several independent clusters. Thus, T4-type phage communities in rice fields consisted of previously uncharacterised members phylogenetically distant from those found in marine environments.

The neighbour-joining phylogenetic tree showed the close relationships of *g23* amino acid sequences from Chinese *g23* clones with those obtained from Japanese rice fields. The clones/phages formed nine clusters (Paddy Groups I to IX) with small clusters of ungrouped paddy clones. Many clones in Northeast China shared groupings with the clones in Japanese rice fields while also showing one Chinese-specific group. In general, there was no tendency for the clones from the specific types of soil and fertilisers, the stage during rice cultivation, or the location, at least within Japan or Northeast China, to belong to the specific Paddy Groups, which indicated that they were not the major factors in determining T4-type phage communities.

Short and Suttle (2005) reported that nearly identical (>99%) sequences of another major capsid gene, *g20*, of phages were recovered from marine and freshwater environments, suggesting that either closely related hosts and the viruses infecting them were distributed widely across environments, or horizontal gene exchange occurs among phage communities in very different environments. Similar clues suggesting the horizontal gene exchange/transfer were obtained in the *g23* sequence analysis. Firstly the identical *g23* sequences at the nucleotide level were observed in two cases among the phages having genome sizes of 60 kb and 160 kb as well as among some phages with isometric and elongated capsids. Furthermore, identical *g23* sequences at the nucleotide level were also observed at distant rice fields. In addition, several cases of identical *g23* sequences at the nucleotide level were observed among the clones obtained from rice fields in Japan and China.

The unrooted phylogenetic tree demonstrated that the majority of *g23* fragments from rice fields (soils and floodwater) formed several clusters independent from those derived from marine environments. The marine groups were distributed narrowly in the tree, which indicated that *g23* genes in paddy fields are more divergent in comparison with those in the marine environment.

In addition, the study on soil depth profiles of *g23* clones to a depth of 1 m indicated that T4-type phage communities of rooting layers were different from those of subsoil layers. This finding suggested that root development may change eubacterial communities in the rooting layers, with resultant changes in *g23* composition (T4-type phage communities). Many uncharacterised *g23* genes and their large diversification as described in this study strongly suggested that rice fields are great genomic reservoirs of viruses.

References

- Ackermann HW (2001) Frequency of morphological phage descriptions in the year 2000. *Archives of Virology* **146**, 843-857.
- Binder B (1999) Reconsidering the relationship between virally induced bacterial mortality and frequency of infected cells. *Aquatic Microbial Ecology* **18**, 207-215.
- Filée J, Tétart F, Suttle CA, Krisch HM (2005) Marine T4-type bacteriophages, a ubiquitous component of the dark matter of the biosphere. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 12471-12476.
- Short CM, Suttle CA (2005) Nearly identical bacteriophage structural gene sequences are widely distributed in both marine and freshwater environments. *Applied and Environmental Microbiology* **71**, 480-486.
- Van Etten, JL, Lane, KC, Meints RH (1991) Viruses and viruslike particles of eukaryotic algae. *Microbiological Reviews* **55**, 586-620.
- Weinbauer MG (2004) Ecology of prokaryotic viruses. *FEMS Microbiology Reviews* **28**, 127-181.
- Wommack KE, Colwell RR (2000) Virioplankton: Viruses in aquatic ecosystems. *Microbiology and Molecular Biology Reviews* **64**, 69-114.