Possible use of viruses as a microbiological agent against harmful algal blooms

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SUMMARY: The ecological importance of aquatic viruses has recently been highlighted. We isolated 2 double-stranded DNA viruses, HaV and HcV, infecting harmful algal bloom (HAB)-causing microalgae, Heterosigma akashiwo and Heterocapsa circularisquama, respectively. We have been investigating their physiological and ecological features especially focusing on the relationships between viruses and host algae. Through cross-infection experiments, it has been strongly suggested that viral infection play an important role in regulating the host population dynamics both quantitatively and qualitatively in the natural environment, especially in the final stage of H. akashiwo blooms. Based on the data accumulated so far, in the present paper, the possible use of the viruses to control HABs is discussed.

KEY WORDS: HABs, virus, Heterosigma akashiwo, Heterocapsa circularisquama, population dynamics, microbiological agent

INTRODUCTION

Heterosigma akashiwo (Raphidophyceae) and Heterocapsa circularisquama (Dinophyceae) are known as HAB (harmful algal bloom)-causing microalgae. H. akashiwo occurs in coastal waters of subarctic and temperate areas of both northern and southern hemispheres, and often causes mortality of cultured fish such as salmon, yellowtail and red sea bream.1,2) H. circularisquama was first recorded in Uranouchi Bay, western Japan, in 1988 and has caused mass mortalities of bivalves such as pearl oysters, oysters and short-necked clams.3-5) Due to the severe damage caused to commercial fisheries, it is important to establish predictive and preventative measures to mitigate the economic losses caused by these blooms.

Algicidal activity of viruses against natural phytoplankton populations has been highlighted6), and now viruses are considered to play an ecologically important role in aquatic environments7). Recently, viruses infecting H. akashiwo and H. circularisquama were isolated from natural seawaters, and designated as HaV8) and HcV9), respectively. To develop practical methods for mitigating harmful algal blooms, the biological characteristics of these viruses have been intensively studied. The present paper overviews the possibility of the practical use of these viruses as microbiological agents against HABs. As far as the authors know, microbiological agents have been only used in terrestrial agriculture, and not in the open ocean. We think that the use of natural microorganisms to control harmful algal blooms has significant potential.

Characteristics of HaV

HaV is a large virus that specifically infects H. akashiwo. It is icosahedral and 202 nm in diameter, and proliferates in the cytoplasm of the host (Fig. 1). In the process of the capsid conformation, empty capsids appear budded from the periphery of the viroplasms, and followed by the insertion of the viral genome. As the viral particles are stainable with DAPI (4', 6-diamidino-2-phenylindole) and the purified viral genome is sensitive to several restriction enzymes, HaV has been shown to contain a double stranded DNA (dsDNA) genome. Although the genetic analysis of HaV-DNA is now under way, we have obtained some data that show a close relationship between HaV and a typical algal virus PBCV-1 which infects a
Fig. 1 (A) Thin section of a healthy *Heterosigma akashiwo* cell showing the nucleus (N), chloroplasts (Ch), mitochondria (M), and Golgi-apparatus (G). (B) Thin section of a *H. akashiwo* cell containing HaV particles. FP, fatty particle; DN, degraded nucleus; VP, viroplasm. (C) Close-up of a negatively-stained HaV particle.

Fig. 2 (A) Thin section of a healthy *Heterocapsa circularisquama* cell showing the nucleus (N), chloroplasts (Ch) and pyrenoids (Py). (B) Thin section of a *H. circularisquama* cell 48 h after inoculation of HcV. (C) Close-up of an intracellular HcV particle.

Fig. 3 A conceptual model describing the interactions between one host algal species and its infectious virus. In the model there are two distinct subpopulations with different characteristics concerning viral infection (A and B). Viruses ΦA and ΦB specifically infect to subpopulations A and B, respectively. For a detailed explanation, refer to Tarutani *et al.* (2000).17
Chlorella-like alga. Based on a one step growth experiment, the burst size and the latent period were estimated as ca. 770 infective viral particles / a lysed cell and 30-33 hr, respectively. 

The host specificity of HaV appears complex. As HaV does not infect another 18 other tested marine microalgal species, it has been concluded to be highly specific to H. akashiwo. However, all HaV strains do not always infect all H. akashiwo strains, i.e., the viral infectivity is considered strain-specific. The mechanism to determine the establishment of infection has not yet been clarified.

HaV was isolated as the first virus hosting a raphidophyte in 1996, and a smaller virus infecting H. akashiwo (HaINV) has recently been isolated. It is most noticeable that HaINV proliferates in the nucleus of H. akashiwo.

Characteristics of HcV

HcV is a large virus that specifically infects H. circularisquama. It is the first virus isolated having a dinoflagellate as a host. The particle is icosahedral and 197 nm in diameter, and proliferates in the cytoplasmic area of the host cell (Fig. 2). In the process of the capsid conformation, empty capsids are inserted with viral genome in the viroplasm-like structure. As the virus particles are stainable with DAPI and the purified genome is sensitive to several restriction enzymes (data not shown), the viral genome is a dsDNA.

As HcV does not infect 24 other tested marine microalgal species, it is considered to be highly specific to H. circularisquama. It has a latent period of 48 to 72 h, and the burst size was estimated to be > 1300 viral particles per lysed cell, based on geometric analysis of thin-sections from infected cells. It is noteworthy that HcV does not eliminate all cells in a host batch culture. About 10% of the cells in the exponentially growing phase survive as immotile and roundish cells that are considered as temporary cysts. When these surviving cells were transferred to a fresh medium and again induced to the exponentially growing phase, they show the sensitivity to HcV infection as the original strain. A mechanism to avoid viral infection could be the formation of a thick-layered envelope like the temporary cyst of H. circularisquama, which is induced by the inoculation of an algicidal bacterium.

Interaction between hosts and viruses in nature

In the natural environments, HaV is detectable in the final stage of H. akashiwo blooms. In 1993, Nagasaka et al. found that the ratio of VLP-harboring cells in a natural population of H. akashiwo specifically increased in the red tide disintegration stage. This data strongly suggested that viral infection impacts on the dynamics of the algal population. Additionally, in 1998, Tarutani et al. found that the clonal composition of a H. akashiwo population was drastically changed by viral infection in the red tide disintegration stage. Based on these data, it is concluded that viral infection has a considerable impact on the host population not only quantitatively but also qualitatively. A hypothetical scheme is shown in Fig. 3.

In contrast, most H. circularisquama clones isolated so far are sensitive to HcV infection. Although HaV is both species-specific and strain-specific, strain-specificity of HcV has scarcely been observed. Considering the broad infectivity of HcV against H. circularisquama, HcV may be a promising tool as a microbiological reagent against HAB. For HaV, preparation of a cocktail of HaV clones would be required, as it is highly strain-specific.

Possible use of HaV and HcV as microbiological agents against harmful algal blooms

It is a concept of a biotic pesticide to control a harmful organism using its natural enemy based on the relationship between the host and the infecting microorganism. In agriculture, some biological agents have already been used practically, and utilization of a natural organism to control pests has been widely studied. By promoting the present research, the possibility of using a natural organism to control a pest in the marine field would be assessed. It has already been clarified that HaV and HcV have several essential characteristics that are required for a microbiological agent

1) Both viruses are specifically infectious to the HAB-causing microalgae.
2) Both viruses were derived from natural seawater and have not been subjected to any genetic manipulation.
3) Both viruses originated from the same place as their host algae.
(4) Both viruses have considerably high growth abilities.
(5) It is possible to produce both viruses at a relatively low cost in the laboratory.

Of course we realize that a considerable amount of preparative work is necessary before any practical use of viruses can be made in the natural field: safety tests against a wider range of organisms, establishment of a large-scale production system, and the development of a preservation technique, etc.

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