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# Influence of a Bacteriophage on the Population Dynamics of Toxic Dinoflagellates by Lysis of Algicidal Bacteria▽†

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**A lytic phage (øZCW1) was isolated from an algicidal bacterium *Pseudoalteromonas* sp. strain SP48 that specifically kills the toxic dinoflagellate *Alexandrium tamarense*. We demonstrated that øZCW1 could trigger the growth of *A. tamarense* by inhibiting the growth of algicidal bacterium SP48. In contrast, the growth of *A. tamarense* was suppressed when cocultured with either SP48 or the øZCW1-resistant mutant of SP48. This study provides the first evidence of the indirect impact of bacteriophage on bloom-forming microalgae via phage lysis of alga-killing bacteria.**

Massive growth of phytoplankton often results in algal blooms in aquatic systems, and blooms of harmful algae can exert negative effects on aquatic ecosystems (12). Although harmful algal blooms (HABs) occur worldwide, the initiation and termination of HABs are still not well understood (2). Bacteria are known to actively interact with HAB species (6, 7). Many algicidal bacteria have been isolated in association with HAB-causing algae, and these bacteria can inhibit the growth of harmful algae (3, 13, 21, 22, 33). The algicidal activity of these bacteria can be influenced by other coexisting organisms in nature, such as competition of nonalgicidal bacteria and prey by heterotrophic protists (14, 15). An unexplored area is the impact of viral lysis on algicidal bacteria and the subsequent (or indirect) effect of such a viral activity on bloom-forming algae.

Viruses are the most abundant biological entities in the aquatic ecosystem, and they often outnumber bacteria by 15-fold (25). Viruses are regarded as one of the major elements in the ocean for regulating biogeochemical cycling, mediating gene transfer, influencing climate change, and modulating community structure (5, 8, 26). Viral infection can account for a significant portion of microbial mortality and is believed to be as important as grazing by protists in keeping microbial biomass in balance (27). Algicidal bacteria become abundant during harmful algal blooms (4, 20). Rapid growth and high cell density of algicidal bacteria make them more vulnerable to phage infection and lysis (29, 32). The lytic activity of phage on algicidal bacteria could reduce the algal lysis activity of bacteria. We hypothesize that phage can influence the formation, growth, and termination of bloom-forming algae by interacting with algicidal bacteria.

A marine bacterium that kills the toxic dinoflagellate *Alexandrium tamarense* was isolated and designated *Pseudoalteromonas* sp. strain SP48 in our previous study (24). *Pseudoalteromonas* sp. strain SP48 produces heat-tolerant algicidal compounds and has a strong algicidal activity against *A. tamarense*. The aim of this study is to investigate the impact of bacteriophage on bloom-forming algae by modulation of the population dynamics of algicidal bacteria.

**Isolation and characterization of bacteriophage and phage-resistant bacterium mutant.** Lytic bacteriophage infecting *Pseudoalteromonas* sp. SP48 was isolated from surface seawater collected from Xiamen Sea, China (24.4°N, 118.1°E) by using the double-layer plaque assay of Adams (1). A bacteriophage that formed clear, round plaques (ca. 2.5 mm in diameter) on strain SP48 was isolated and designated ϕZCW1. The morphology of ϕZCW1 was examined using a JEM2100 transmission electron microscope (TEM). ϕZCW1 is a myovirus with an isometric head (ca. 123 nm in diameter) and a long, contractile tail (ca. 235 nm long) (Fig. 1). ϕZCW1 is a double-stranded DNA virus, and it has a latent period of ca. 1.5 h and a burst size of ca. 91 (see Fig. S1 in the supplemental material).

Twenty bacterial strains were used to test the host specificity of ϕZCW1 (Table 1). Susceptibility of bacteria to ϕZCW1 was determined by the appearance of plaques on double-layer agar plates. ϕZCW1 caused lysis of only *Pseudoalteromonas* sp. SP48 and not the other bacterial strains tested (Table 1), demonstrating the high host specificity of this phage.

A ϕZCW1-resistant SP48 mutant, SP48-M, was obtained from a bacterial colony recovered from a clear plate by the plaque assay by the method of Huang et al. (11). The phage-resistant activity of SP48-M was sustained for several hundred generations.

**Phage-bacterium-microalga assemblies.** In order to understand the interactions between the HAB species *A. tamarense*, algicidal bacterium SP48 (that specifically kills *A. tamarense*), and the lytic phage ϕZCW1 (that specifically kills SP48), different assemblages of these microbial consortia were established (Table 2). Experiment 1 contained 100 ml of exponentially growing *A. tamarense* culture (ca. 13,000 cells $ml^{-1}$). For experiments 2, 3, and 4, exponentially growing bacterial cultures were washed in f/2 medium (9) by centrifugation (3,000 ×

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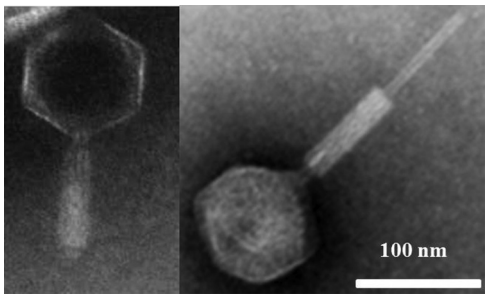


FIG. 1. Micrographs of phage ϕZCW1 with a contractile tail (left panel) and contracted tail (right panel) as viewed in a JEM2100 transmission electron microscope.

*g*, 15 min) three times to remove extracellular algicidal compounds. Experiment 2 included the mixture of 100 ml of exponentially growing *A. tamarense* (ca. 13,000 cells $ml^{-1}$) and SP48 (ca. $4.7 \times 10^7$ cells $ml^{-1}$). For experiments 3 and 4, washed bacterial cultures, strains SP48 (ca. $4.9 \times 10^7$ cells $ml^{-1}$) and SP-M (ca. $1 \times 10^8$ cells $ml^{-1}$), were mixed with bacteriophage ϕZCW1 separately with a phage/bacterium ratio of 1:10. The mixture was added to 100 ml exponentially growing *A. tamarense*. Aliquots (10 ml) of Zobell 2216E medium (BD Bioscience) were added to all four assemblages to support the growth of bacteria (24). Each experiment was carried out in triplicate. Axenic *A. tamarense* ATGD98-006 cultures used in this study were obtained by removing free-living and attached bacterial symbionts using detergents and antibiotics (23). The axenic cultures were grown in f/2 medium (without silicate) at 20°C on a 12-h light-dark cycle (12-h light–12-h dark) with a photo flux density of 50 μmol $m^{-2}$ $s^{-1}$. The algicidal activity of *Pseudoalteromonas* sp. SP48 and lytic activity of bacteriophage ϕZCW1 were evaluated by microscopic counting of bacterial cells and virus-like particles, respectively, following the protocol of Patel et al. (19). To count *A. tamarense*, samples were fixed with Lugol's iodine followed by direct microscopic counting (24).

TABLE 1. ϕZCW1 host range assay and bacterial strains used in this study

| Bacterial strain tested | Algicidal activity[a] | Geographic origin of strain | Susceptibility[b] |
|---|---|---|---|
| *Pseudoalteromonas* sp. SP48 | + | East China Sea | + |
| *Alteromonas* sp. SP31 | + | East China Sea | − |
| *Alteromonas* sp. SP44 | + | East China Sea | − |
| *Pseudoalteromonas* sp. DHQ17 | + | East China Sea | − |
| *Pseudoalteromonas* sp. DHQ4 | + | East China Sea | − |
| *Pseudoalteromonas* sp. DHQ5 | + | East China Sea | − |
| *Pseudoalteromonas* sp. DHY3 | + | East China Sea | − |
| *Pseudoalteromonas* sp. HH2 | + | East China Sea | − |
| *Pseudoalteromonas* sp. HH5 | + | East China Sea | − |
| *Idiomarina* sp. SP96 | + | East China Sea | − |
| *Alteromonas* sp. DH46 | + | East China Sea | − |
| *Vibrio* sp. DH51 | + | East China Sea | − |
| *Halomonas* sp. DH77 | + | East China Sea | − |
| *Marinobacter* sp. CN46T | + | South China Sea | − |
| *Marinobacter* sp. CN74T | + | South China Sea | − |
| *Marinobacter* sp. B17 | − | East China Sea | − |
| *Marinobacterium* sp. AN9 | − | South Korea | − |
| *Marinobacterium* sp. KCTC12756 | − | Deokjeok Island, South Korea | − |
| *Marinobacterium* sp. F19 | − | East China Sea | − |
| *Novosphingobium* sp. F2 | − | East China Sea | − |

[a] Symbols: +, with algicidal activity; −, without algicidal activity.
[b] Symbols: +, sensitive to viral infection; −, resistant to viral infection.

TABLE 2. Different assemblage setups for experiments

| Expt | Harmful alga | Bacteria | Bacteriophage |
|---|---|---|---|
| Expt 1 | *A. tamarense* | | |
| Expt 2 | *A. tamarense* | *Pseudoalteromonas* sp. SP48 | |
| Expt 3 | *A. tamarense* | *Pseudoalteromonas* sp. SP48 | ϕZCW1 |
| Expt 4 | *A. tamarense* | *Pseudoalteromonas* sp. SP48-M | ϕZCW1 |

In experiment 1, dinoflagellate *A. tamarense* grew from 13,600 to 38,566 cells $ml^{-1}$ in 72 h (Fig. 2). The growth of *A. tamarense* was inhibited and decreased from 13,600 cells $ml^{-1}$ to an undetectable level in 72 h in experiment 2 (with algicidal bacterium SP48). The growth inhibition of *A. tamarense* by strain SP48 was reduced in experiment 3 (with SP48-specific phage). The cell density of *A. tamarense* in experiment 3 increased slightly in 36 h and decreased to 8,866 cells $ml^{-1}$ in 72 h. In experiment 4, the growth of *A. tamarense* was inhibited and decreased to an undetectable level in 72 h with the addition of phage-resistant bacterium SP48-M (Fig. 2).

Bacterial abundance in experiment 2 increased from $4.7 \times 10^7$ to $1.1 \times 10^{10}$ cells $ml^{-1}$ at 60 h (Fig. 3). In contrast, addition of phage ϕZCW1 in experiment 3 inhibited the growth of algicidal bacterium SP48 (Fig. 3). The bacterial abundance stabilized at $10^7$ cells $ml^{-1}$, whereas the number of bacteria reached $1 \times 10^{10}$ cells $ml^{-1}$ when bacteriophage was not added. The ϕZCW1-resistant bacterium SP48-M was able to grow (inset in Fig. 3) in experiment 4 but to a lower degree than in experiment 2, which tested *A. tamarense* and SP48 but no phage. Abundance of phage ϕZCW1 increased from $1.4 \times 10^6$ to $2.7 \times 10^8$ particles $ml^{-1}$ in experiment 3 during the first 12 h and remained relatively stable within 72 h (Fig. 4). Viruses could not be enumerated in experiment 4, where the mutant host strain SP48-M was mixed with phage ϕZCW1.

Lytic viruses are believed to play an important role in regulating the microbial abundance and distribution via the "kill-the-winner" model (28, 31). Viruses actively interact with heterotrophic bacteria, cyanobacteria, and eukaryotic algae and are capable of regulating population structure and genetic diversity of bacteria and microalgae in the marine environment (17, 18, 30). However, little is known about the potential impact of phage-host interaction on the population dynamics of eukaryotic microalgae. We demonstrated that bacteriophage can influence the growth of eukaryotic microalgae by killing the bacteria that are lethal to algae. In the mixture of *A. tamarense*, *Pseudoalteromonas* sp. SP48, and the lytic phage ϕZCW1 (experiment 3), ϕZCW1 was able to reduce the algicidal activity of strain SP48 by killing the host bacteria. The inhibition of algal growth was relaxed at the first 36 h due to the active viral lysis of SP48. A dramatic increase of viral particles in experiment 3 further supports the lytic activity of ϕZCW1. Unexpectedly, algal abundance showed a slight decrease after 36 h. This might be caused by development of a *Pseudoalteromonas* sp. SP48 mutant strain resistant to ϕZCW1. Bacterial strains resistant to viruses can be an important factor in affecting the lytic efficiency of viruses. On the other hand, when ϕZCW1-sensitive bacterium SP48 was replaced with ϕZCW1-resistant bacterium SP48-M (experiment

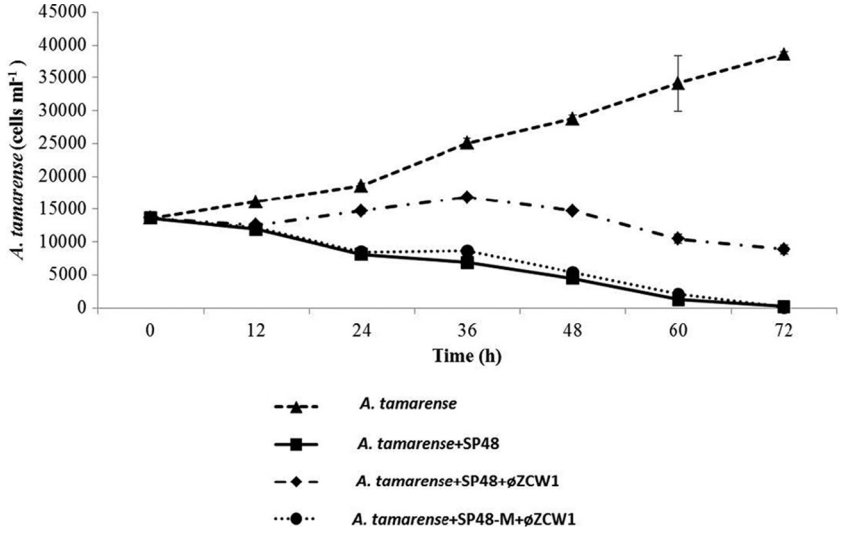


FIG. 2. Population dynamics of harmful alga *A. tamarense* in different assemblages. *A. tamarense* represents growing *A. tamarense* culture without any additions. *A. tamarense*+SP48 represents coculture of *A. tamarense* and bacterium *Pseudoalteromonas* sp. strain SP48. *A. tamarense*+SP48+ϕZCW1 represents coculture of *A. tamarense* and bacterium *Pseudoalteromonas* sp. SP48 and bacteriophage ϕZCW1. *A. tamarense*+SP48-M+ϕZCW1 represents coculture of *A. tamarense* and bacterium *Pseudoalteromonas* sp. SP48-M and bacteriophage ϕZCW1. Each experiment was carried out in triplicate. The error bars represent the standard deviations for the values at different time points.

4), the cell numbers of *A. tamarense* decreased to an undetectable level after 72 h of coincubation of these three microbial consortia. In this case, strain SP48-M was able to kill *A. tamarense* because SP48-M is resistant to phage ϕZCW1. The biomass of SP48-M was lower than the biomass of SP48 in experiment 2. The growth rate of phage-resistant bacterial mutant is in general significantly lower than that of the wild type (10). Obvious reduced growth capability of phage-resistant strains has been detected in some other studies (16). However, SP48-M still showed great algicidal activity against *A. tamarense* and activity as great as that of *Pseudoalteromonas* sp. SP48. This is ascribed to the algicidal mechanism of *Pseudoalteromonas* sp. SP48, which secretes algicidal compounds.

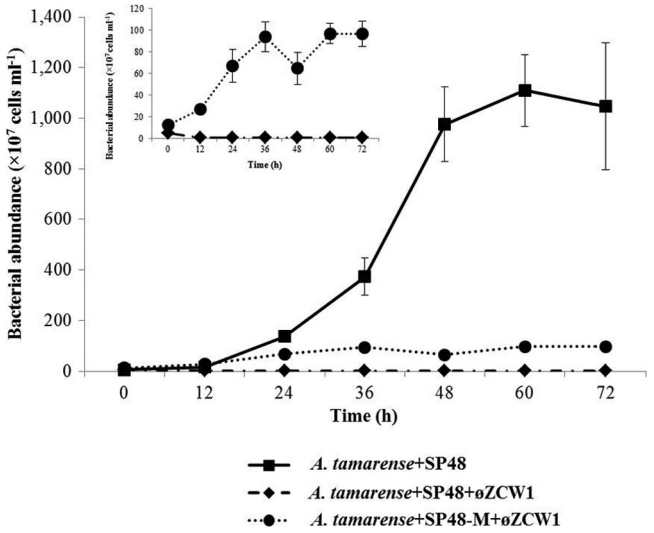


FIG. 3. Population dynamics of algicidal bacterium *Pseudoalteromonas* sp. SP48 and phage-resistant bacterium mutant SP48-M in different assemblages. *A. tamarense*+SP48 represents coculture of *A. tamarense* and bacterium *Pseudoalteromonas* sp. SP48. *A. tamarense*+SP48+ϕZCW1 represents coculture of *A. tamarense* and bacterium *Pseudoalteromonas* sp. SP48 and bacteriophage ϕZCW1. *A. tamarense*+SP48-M+ϕZCW1 represents coculture of *A. tamarense* and bacterium *Pseudoalteromonas* sp. SP48-M and bacteriophage ϕZCW1. Each experiment was carried out in triplicate. The error bars represent the standard deviations for the values at different time points.

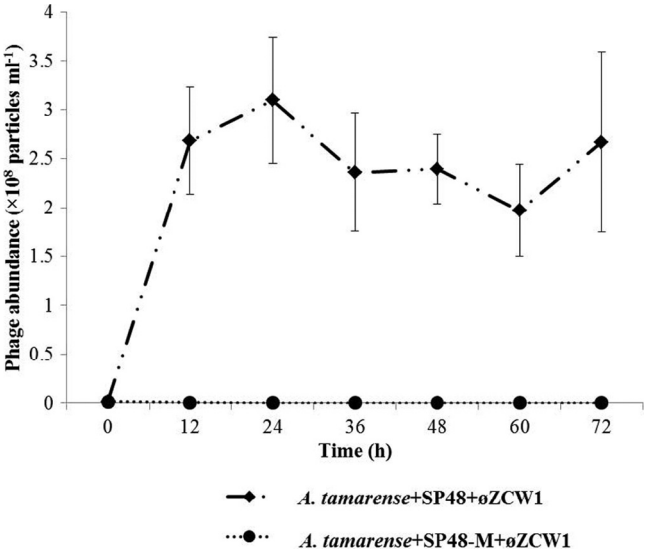


FIG. 4. Population dynamics of bacteriophage ϕZCW1 in different assemblages. *A. tamarense*+SP48+ϕZCW1 represents coculture of *A. tamarense* and bacterium *Pseudoalteromonas* sp. SP48 and bacteriophage ϕZCW1. *A. tamarense*+SP48-M+ϕZCW1 represents coculture of *A. tamarense* and bacterium *Pseudoalteromonas* sp. SP48-M and bacteriophage ϕZCW1. Each experiment was carried out in triplicate. The error bars represent the standard deviations for the values at different time points.

When the bacterial concentration is high enough to produce sufficient accumulation of algicidal compounds, the algicidal activity shows a less direct relationship to bacterial abundance.

In short, our results suggest that viruses may influence nonhost microbes through a "chain reaction" mechanism. By lysing specific hosts that are lethal to other microorganisms, viruses can influence microbial population dynamics indirectly. Such a chain reaction caused by phage or viruses has not been tested in the natural environment. The phage-bacterium-alga model may be a good system to study the ecological interactions between phage, bacteria, and algae to provide a better understanding of the formation and termination of algal blooms in aquatic systems.

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