

Lysogeny and Lytic Viral Production during a Bloom of the Cyanobacterium *Synechococcus* spp.

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ABSTRACT

Lytic viral production and lysogeny were investigated in cyanobacteria and heterotrophic bacteria during a bloom of *Synechococcus* spp. in a pristine fjord in British Columbia, Canada. Triplicate seawater samples were incubated with and without mitomycin C and the abundances of heterotrophic bacteria, cyanobacteria, total viruses and infectious cyanophage were followed over 24 h. Addition of mitomycin C led to increases in total viral abundance as well as the abundance of cyanophages infecting *Synechococcus* strain DC2. Given typical estimates of burst size, these increases were consistent with 80% of the heterotrophic bacteria and 0.6% of *Synechococcus* cells being inducible by the addition of mitomycin C. This is the highest percentage of lysogens reported for a natural microbial community and demonstrates induction in a marine *Synechococcus* population. It is likely that the cyanophage production following the addition of mitomycin C was much higher than that titered against a single strain of *Synechococcus*; hence this estimate is a minimum. In untreated seawater samples, lytic viral production was estimated to remove ca. 27% of the gross heterotrophic bacterial production, and a minimum of 1.0% of the gross cyanobacterial production. Our results demonstrate very high levels of lysogeny in the heterotrophic bacterial community, outside of an oligotrophic environment, and the presence of inducible lysogens in *Synechococcus* spp. during a naturally occurring bloom. These data emphasize the need for further examination of the factors influencing lytic and lysogenic viral infection in natural microbial communities.

Introduction

Viruses are important mortality agents of marine microbes and consequently can affect nutrient cycling, pop-

ulation abundances, community diversity, and, potentially, genetic transfer (reviewed in [5, 31, 20, 35]). The effect of viruses on microbial communities and chemical cycling depends on whether the virus is lytic or temperate. Lytic viral infection in a population results in continual infection and cell lysis and therefore continuous

production of viruses. Temperate viruses incorporate into the host genome and result in viral production only following an induction event.

Lysogeny may be advantageous for viruses when host abundances are low and the likelihood of a virus contacting a sensitive host is reduced [29, 15]. A temperate virus overcomes this limitation because once incorporated it is replicated along with the host genome. Consequently lysogeny can provide a refuge for viruses during times when bacterial abundance is too low to sustain a lytic infection. Lysogenized cells may also benefit by gaining resistance to infection by other, similar lytic viruses [17], or by acquiring beneficial genes carried by the virus [25].

A wide range of values have been reported for the proportion of lysogenized cells in studies with marine bacterial isolates [12, 14] as well as natural communities [2, 13, 27, 28, 30]. Estimates of the percentage of lysogenized cells obtained in these studies vary from about 0.07 to 43.3% [12, 27]. It has been suggested that this wide range is because of the effects of trophic state on microbial communities, with eutrophic environments generally having a lower percentage of lysogens than oligotrophic communities [12, 14, 28]. However, Jiang and Paul [13] found the highest percentages of lysogens in a eutrophic estuary.

Most studies of lysogeny have focused on heterotrophic bacteria, even though lysogeny is known to occur in filamentous cyanobacteria (reviewed in [21]). There is much less evidence for lysogeny in unicellular coccoid cyanobacteria, although Sode et al. [16] discovered a temperate cyanophage in a strain of *Synechococcus* spp. and Wilson and coworkers have argued for its existence in isolates and populations of marine *Synechococcus* spp. [32, 33, 34]. A bloom of *Synechococcus* spp. encountered during a research cruise provided an opportunity to investigate the occurrence of lysogeny and the contribution of viral lysis to mortality in natural marine communities of *Synechococcus* spp. and heterotrophic bacteria.

Materials and Methods

Location and Sampling

This experiment was conducted during a research cruise in the Strait of Georgia, British Columbia, Canada, during August 2000. At the entrance to Squirrel Cove, Cortez Island (50° 07,318' N, 124° 54.264' W) a 10-L Go-Flo bottle mounted on a rosette with a CTD and fluorometer was used to collect natural microbial communities from the chlorophyll maximum at 8.5 m during a bloom of *Synechococcus* spp.

Incubations

Whole seawater was divided into six 250 ml subsamples in polyethylene bags (Whirl-Pac). Three subsamples were not modified and served as controls, while the other three bags had mitomycin C (Sigma M-0503) added to a final concentration of 1 $\mu\text{g mL}^{-1}$; this concentration has been found to be most effective for inducing lysogens [27]. Incubations were conducted in a flowing seawater bath that maintained *in situ* temperature throughout the experiment. One layer of neutral-density screening placed over the water bath reduced incident solar radiation by 33%.

Direct Counts

Immediately following the addition of mitomycin C and at approximately 6-h interval afterwards for 24 h, the bags were sampled to determine abundances of viruses, heterotrophic bacteria, cyanobacteria, and cyanophages. For direct counts of viruses and heterotrophic bacteria, 200 μL of water was collected, immediately diluted with virus-free water, and stained with Yo-Pro-1 (Molecular Probes, Eugene, OR) according to Hennes and Suttle [10]. Bacterial abundances determined by DAPI and Yo-Pro staining are not significantly different (unpublished data). To determine the abundance of cyanobacteria, 1 mL of water was sampled, filtered onto a 0.2 μm pore-size black polycarbonate filter (Millipore), and mounted on a slide with immersion oil. All prepared slides were kept frozen at -20°C until they were counted 1 month later.

Viruses, heterotrophic bacteria and cyanobacteria were counted using an Olympus AX70 epifluorescence microscope. In all cases, a minimum of 200 particles in at least 20 random fields were counted. Yo-Pro-stained slides were illuminated with a wide-blue excitation filter (450–480 nm). Virus particles were enumerated first, followed by heterotrophic bacteria to minimize fading of stained viruses. Cyanobacteria were counted using a wide-green excitation filter (510–550 nm). Counts were converted to particles mL^{-1} as outlined in Suttle [18].

MPN Assay

To determine the abundance of infectious cyanophages, 5 mL was collected at each time point and filtered through a 0.45 μm nominal pore size PVDF (Durapore, Millipore) syringe filter to remove host cells. The filtrate was then stored at 4°C in the dark until most probable number (MPN) assays were performed, within 3 months, as outlined in Suttle and Chan [23]. Infectious cyanophage concentrations are stable in samples stored over this time period (Rodda and Suttle, unpublished data).

Synechococcus strain DC2 (a.k.a. WH 7803) was used as a host for all assays and was grown under continuous light at 21°C in artificial seawater (33 psu) [8] modified by the addition of Se [3]. This strain has been shown to be permissive to infection by a large number of different cyanophage and consequently is frequently used to estimate cyanophage abundance in natural

samples [22, 23, 26]. The MPN assays were performed in 96-well microtiter plates. For each sample, 10-fold dilutions of filtrate were added to exponentially growing hosts with 16 replicates at each dilution. The plates were incubated under the same conditions as the host batch cultures for 10 to 14 d. After this time, wells showing no pigmentation were scored as positive for the presence of a lytic agent. These scores were converted to abundances of lytic agents using the MPN program by Hurley and Roscoe [11].

Growth Rate Calculations

Net production was calculated based on changes in cell numbers over the first 18 h of the incubation. During this time, growth of heterotrophic bacteria and cyanobacteria occurred in the control and mitomycin C treatments. Changes in cell numbers were converted to growth rates based on the following:

$$\left(\frac{n_2 - n_0}{t_2 - t_0}\right) \div n_0 \times 24$$

n = number of cells
 t = time

To calculate the percentage of production lost through viral lysis, gross production in the absence of lysis was calculated at 12 h, which is when virus abundance increased in control treatments. Gross production was calculated by adding the number of cells lysed to produce the observed increase in virus abundance to the net production at 12 h.

$$\frac{n_1 + \left(\frac{v_1 - v_0}{\text{Burst size}}\right) - n_0}{t_1 - t_0}$$

Gross production therefore represents estimated production in the absence of viral lysis, but may include other loss factors such as grazing.

Results and Discussion

Heterotrophic Bacteria and Cyanobacteria

The presence of an obvious bloom of *Synechococcus* spp. provided a special opportunity to investigate inducible lysogens in the bloom, as well as viral mediated mortality. The temperature and salinity at the depth of sampling (8.5 m) was 16.6°C and 25.1 psu. Despite the bloom conditions, the abundances of viruses and bacteria (10^8 mL⁻¹ and 10^7 mL⁻¹, respectively) were within the ranges reported for other coastal studies [35] and were similar to others detected in the Strait of Georgia during blooms (unpublished data).

In the untreated samples, growth of heterotrophic bacteria (Fig. 1A) and cyanobacteria (Fig. 2A) continued over the first 18 h of the incubations, suggesting that

growth was not nutrient limited. Average net growth rate of the heterotrophic bacteria in the controls, based on changes in bacterial numbers, was 4.82 d⁻¹ (SD = 0.64, $n = 3$), while the cyanobacteria had an average net growth rate of 0.66 d⁻¹ (SD = 0.21, $n = 3$).

In both the heterotrophic bacteria and cyanobacterial communities, growth rate was reduced in the mitomycin-C treatments. Estimates of average net growth rates were 0.68 d⁻¹ (SD = 0.54, $n = 3$) for heterotrophic bacteria and 0.008 d⁻¹ (SD = 0.27, $n = 3$) for cyanobacteria. Decreased growth in the mitomycin-C treatments was likely the result of the induction of lysogens, resulting in cell lysis and damage to DNA [7].

Lytic Viral Production

Measurable lytic viral production occurred in the controls for 12 h after the start of the incubations. This was true for both total viruses (Fig. 1B) and infectious cyanophages (Fig. 2B). Combining the net heterotrophic bacterial production (4.80×10^5 cells mL⁻¹ h⁻¹ (SD = 5.66×10^4 , $n = 3$)) and the number of cells lysed by viruses, the gross heterotrophic bacterial production can be estimated. Assuming the total virus community was predominately bacteriophage, and a burst size of 50 [9], the gross heterotrophic bacterial production in the absence of viral lysis was estimated to be 6.60×10^5 cells mL⁻¹ h⁻¹ (SD = 4.77×10^4 , $n = 3$). Therefore, viral lysis was responsible for removal of ca. 27% (SD = 4, $n = 3$) of the gross heterotrophic productivity. Other authors have used a smaller burst size [27, 28], but given the bloom situation and high bacterial growth rates the larger burst size seems warranted. This is similar to estimates from other studies that suggest that viruses are important mortality agents for bacterial communities (reviewed in [1, 19, 5, 31, 35]).

In comparison, the average gross cyanobacterial production was 3.79×10^3 cyanobacteria mL⁻¹ h⁻¹ (SD = 8.43×10^2 , $n = 3$) with lytic virus production, assuming a burst size of 81 [24], causing lysis of 1.0% (SD = 0.4, $n = 3$) of gross cyanobacterial production. However, the abundance estimates of lytic viruses and inducible lysogens are minimums for several reasons.

In order to distinguish cyanophages from total viruses it was necessary to use MPN assays. These provided minimum estimates of cyanophage abundance because they only detect viruses that infect a specific host strain. Nonetheless, MPN assays have been widely used to esti-

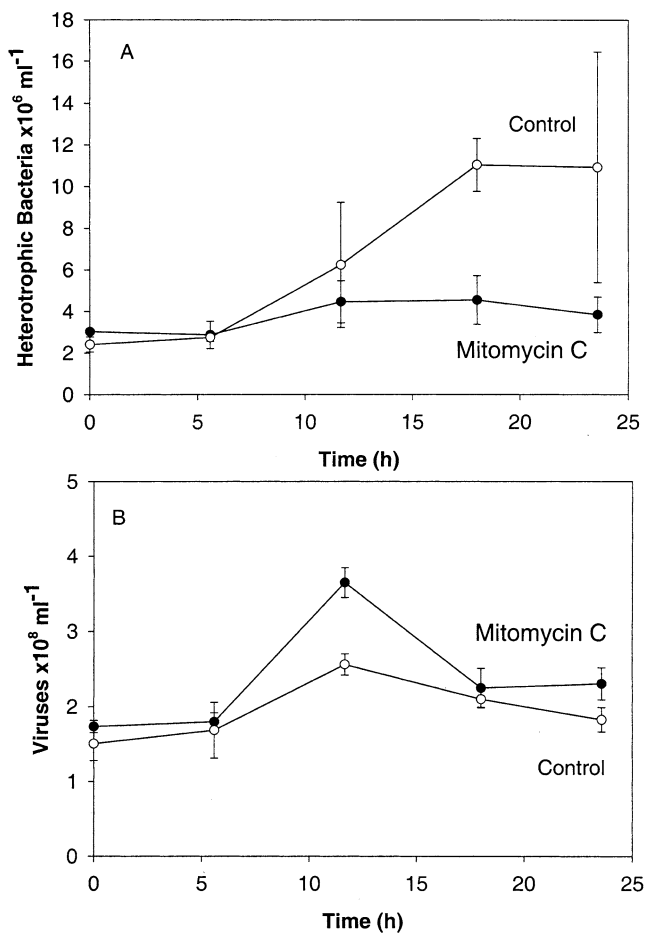


Fig. 1. Abundances of (A) heterotrophic bacteria and (B) total viruses in untreated (○) and mitomycin-C treated (●) samples over the course of the experiment. Values given are means of triplicate incubations with standard deviations.

mate the abundance of cyanophages infecting *Synechococcus* spp. (reviewed in [35]). We choose *Synechococcus* strain DC2 as an assay organism because it is susceptible to infection by many cyanophages [22]. However, all cyanophages that infect marine *Synechococcus* do not infect DC2. Moreover, DC2 is a subtropical isolate and may not be as good a host for cyanophages from temperate waters. Finally, filtration to remove host cells may have decreased cyanophage concentrations.

Induction of Lysogens

Similar to the controls, total virus abundances increased in the mitomycin C treatments over the first 12 h (Fig. 1B), while abundances of infectious cyanophage continued to increase until 18 h (Fig. 2B). Elevation of virus abundance

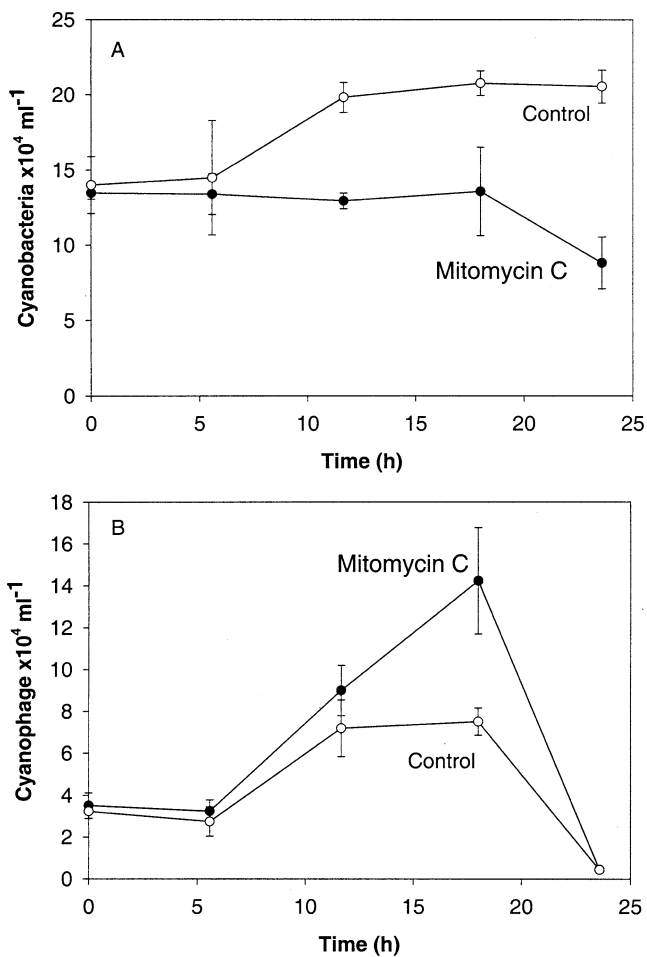


Fig. 2. Abundances of (A) cyanobacteria and (B) infectious cyanophage in untreated (○) and mitomycin-C treated (●) samples over the course of the experiment. Values given are means of triplicate incubations. Errors for the cyanobacteria are standard deviations while those for the cyanophage are standard errors.

in the mitomycin C treatments was considered to be the result of induction of lysogens.

Total virus production in mitomycin C treatments was elevated compared to controls, with an average of 1.08×10^8 mL⁻¹ more virus being produced in the treated bags. If a burst size of 50 viruses per cell is assumed, this is equivalent to the lysis of 2.17×10^6 cells mL⁻¹, corresponding to the induction by mitomycin C of 80% of the heterotrophic bacteria present at the beginning of the incubation.

Cyanophage abundance after the addition of mitomycin C was not significantly different from the control up to 12 hours after treatment and therefore was considered lytic production. Lysogenic production was estimated from the difference in cyanophage abundance (6.74×10^4 cyanophage mL⁻¹) between the mitomycin C treatments and

the controls after 18 h. The difference in infectious cyanophage abundance is estimated to have resulted from the induction of lysogens in 8.32×10^2 cyanobacteria mL^{-1} , or 0.6% of the cyanobacteria. Again, this only accounts for cyanophage that were produced that can be titered on *Synechococcus* strain DC2.

Because not all lysogens can be induced with mitomycin C, the incubations in this study provide a method of estimating the minimum percentage of bacteria in a community that are lysogens. Natural induction events are poorly understood, but two possible inducing agents, sunlight and high growth rate, were present during the incubations. In the present study, mitomycin C clearly induced lysogens. Lysis of 80% of the heterotrophic bacteria present at the start of the incubation is attributed to lysogen induction. Previous studies of natural microbial communities have found as few as 0.07% of cells to be inducible by mitomycin C [27], whereas studies using bacterial isolates have found that up to 50% of cells could be induced [12]. The estimates from our study are much higher than previous estimates for most marine systems. However, according to Freifelder [4], as many as 90% of phages are temperate. This would suggest that very high levels of lysogeny are possible in temperate coastal seawater.

In contrast to the results for heterotrophic bacteria, the cyanobacteria appeared to be less affected by viral infection. The production rate of viruses infecting *Synechococcus* strain DC2 was much lower than reported from the Gulf of Mexico [6, 23]. In the Gulf of Mexico, however, the ratio of cyanophage to cyanobacteria was much higher than reported here. The ratios found in this study (0.25) were more similar to those reported for Woods Hole, MA, by Waterbury and Valois [26], who argued that most cyanobacteria were resistant to co-occurring cyanophage. With low rates of contact between hosts and viruses, lysogeny may be a beneficial strategy. However, under the conditions present in this study, we did not find evidence of a high proportion of inducible lysogens.

This is the first report simultaneously investigating the impact of viral lysis and lysogen induction in different components of a marine microbial community. Estimates of viral lysis based on incubations of whole seawater showed that a large percentage of the gross heterotrophic bacterial production was lost through viral lysis, whereas there was less evidence that cyanophage had a large impact on gross cyanobacterial production. The effects of lysogeny were less clear. Although the majority of the hetero-

trophic bacterial standing stock contained inducible lysogens, the effects of these viruses on the *in situ* bacterial community are unknown. Under natural conditions, only a small percentage of lysogens are likely to be induced at any given time, resulting in a minor effect on microbial mortality. As only a small percentage of *Synechococcus* cells were clearly induced by mitomycin C, temperate viruses may have had less effect on cyanobacterial mortality. Alternatively, mitomycin C may not be an effective inducing agent for lysogenic cyanophage. For example, previous studies have shown that heavy metals induce a cyanophage that infects *Synechococcus* sp. [16].

Previous evidence suggests that in a bloom situation lytic viral production should be high, with little evidence of lysogens [12, 14, 28]. In our study, lytic infection was important in the lysis of heterotrophic bacteria but was less evident in cyanobacteria.

Unexpectedly, a high percentage of heterotrophic bacteria were lysogens, suggesting that either the state of the environment or the community itself favors lysogeny over lytic viral production. There was less evidence that the cyanobacterial community contained a high percentage of lysogens. This suggests that the trophic state of the environment is not a good predictor of the relative impacts of viral lysis and lysogeny on the microbial community. Alternatively, we may have greatly underestimated the proportion of cyanobacterial lysogens because of biases in the MPN assay, or our inability to cause induction with mitomycin C. Nonetheless, because the benefits of each type of replication are dependent on the relative abundance of the virus and sensitive host, the composition of the microbial community is likely an important factor affecting the prevalence of lysogeny.

Our results extend previous findings by showing that *Synechococcus* populations during a bloom contain inducible temperate phage, and that a very high percentage of marine heterotrophic bacteria in the same community are also lysogenized. Further studies are needed to identify natural inducing agents and determine the role lysogeny plays in the abundance and genetic diversity of marine microbial communities.

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