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Effect of Nutrient Addition and Environmental Factors on Prophage Induction in Natural Populations of Marine *Synechococcus* Species

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A series of experiments were conducted with samples collected in both Tampa Bay and the Gulf of Mexico to assess the impact of nutrient addition on cyanophage induction in natural populations of *Synechococcus* sp. The samples were virus reduced to decrease the background level of cyanophage and then either left untreated or amended with nitrate, ammonium, urea, or phosphate. Replicate samples were treated with mitomycin C to stimulate cyanophage induction. In five of the nine total experiments performed, cyanophage induction was present in the non-nutrient-amended control samples. Stimulation of cyanophage induction in response to nutrient addition (phosphate) occurred in only one Tampa Bay sample. Nutrient additions caused a decrease in lytic (or control) phage production in three of three offshore stations, in one of three estuarine experiments, and in a lysogenic marine *Synechococcus* in culture. These results suggest that the process of cyanophage induction as an assay of *Synechococcus* lysogeny was not inorganically nutrient limited, at least in the samples examined. More importantly, it was observed that the level of cyanophage induction (cyanophage milliliter^{-1}) was inversely correlated to *Synechococcus* and cyanophage abundance. Thus, the intensity of the prophage induction response is defined by ambient population size and cyanophage abundance. This corroborates prior observations that lysogeny in *Synechococcus* is favored during times of low host abundance.

Bacteriophages are excellent survivors, having evolved different strategies for coping with environmental challenges. In addition to the familiar lytic infection, certain temperate phages can alternatively become integrated into the host chromosome as a prophage. Lysogeny has been extensively studied in heterotrophic bacteria. The most well known example is the *Escherichia coli* (λ) system, which has been studied in detail for over 51 years, accounting for much of what is known today about the molecular basis of the interactions between temperate phage and their host.

An extensive survey of cultured, heterotrophic bacteria estimated that approximately 50% of bacterial strains were lysogenic (1). Recent sequencing of bacterial genomes has also conclusively demonstrated that integrated viral genomes are common. A total of 51 of 82 genomes examined were determined to carry prophages, and within those 51 genomes, a total of 230 recognizable putative prophages were identified (4).

Initially, lysogeny was believed to be of limited importance in marine environments (reviewed in reference 12). However, it has been demonstrated experimentally that a large number of heterotrophic marine bacterial strains contain inducible prophage (11, 12). Furthermore, studies have demonstrated temporal variations in the prevalence of lysogeny in heterotrophic bacterial populations (7, 38).

Lysogeny has been described as an adaptation of viruses allowing for survival during adverse conditions, especially when there is low host abundance. Mathematical modeling has demonstrated that there is a reciprocal relationship between bacterial diversity and viral abundance, with lysogeny theorized to act as the repository preventing viruses from disappearing altogether during periods of low host abundance (30).

In addition to heterotrophic bacterioplankton, the marine environment contains many autotrophic prokaryotes. Since they were first described in 1977, it has become increasingly evident that cyanobacterial picoplankton play an essential ecological role in the marine environment. These picoplankton are distributed throughout the oceans, except for the polar regions, and are most abundant in tropical and temperate waters. It has been estimated that the *Synechococcus* component of the picoplankton may account for up to 25% of worldwide primary productivity and up to 60% of the total primary productivity at the 1% light level (33).

Despite the obvious ecological importance of picoplankton, there has been very little research to date on lysogeny in these autotrophic organisms. Much of the investigation to date on cyanophage and cyanobacteria has focused on lytic interactions and characterization of cyanophage. Understanding lytic interactions between cyanophage and cyanobacteria is important because viral infection has been found to be a significant factor in the mortality of cyanobacteria. Viral lysis is responsible for a large but variable fraction of mortality in cyanobacteria, estimated to be on the order of 30% (22). Examination of natural cyanobacterial samples has shown that up to 5% of cyanobacteria contain mature phage (22).

Viruses have also demonstrated the potential to decrease primary productivity in phytoplankton populations. The addition of seawater concentrates of the 0.002- to 0.2-μm-size fraction to phytoplankton cultures resulted in an average decline in primary productivity of 44% (29). In these experiments, *Synechococcus* species were much less sensitive than other types of phytoplankton to the inhibitory effect of viral concentrates (27). It is possible that this increased resistance to phage pressure is due to the presence of homoimmunity resulting from

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lysogeny in the cyanobacterial community. The occurrence of lysogeny in this ubiquitous phytoplankton may have important consequences in carbon fixation and microbial loop processes.

Early research verified lysogeny in the freshwater cyanobacterium *Plectonema boryanum* (23). These cyanobacterial isolates demonstrated spontaneous induction of prophage as well as induction when treated with mitomycin C or glutathione (3). Lysogeny has also been reported in the marine filamentous form *Phormidium* sp. (18). Some preliminary evidence also suggests that lysogeny in cyanobacteria may have a molecular basis similar to that of heterotrophic organisms (20). At this time, it is unknown how widespread lysogeny may be in autotrophic prokaryotes.

Cyanophages that infect marine *Synechococcus* strains have been confirmed to have a very high genetic diversity (reviewed in reference 12). In addition, this overall species richness was observed to be higher in the winter and lowest in the summer, despite higher overall abundance in the summer. This seasonal variation in richness of genetic types may be partially due to the presence or absence of naturally occurring prophage induction in *Synechococcus.*

It has recently been demonstrated that lysogeny occurs in natural populations of marine *Synechococcus* (14, 19). We have previously found a seasonal pattern in the occurrence of lysogeny in *Synechococcus*, with a prevalence of positive inductions in late winter to early spring (14).

In addition to prophage induction in natural populations, induction of cyanophage has been demonstrated from a cultured marine *Synechococcus* species isolated from the coastal waters off of Kyushu, Japan. Cultures of this cyanobacterial isolate were shown to produce prophage when induced by UV light, mitomycin C (25), and a heavy metal (24). There has also been a report of mitomycin C-stimulated prophage induction in cultured and natural samples of the filamentous cyanobacterium *Trichodesmium* sp. (17).

The factors controlling the switch from lysogenic to lytic existence in cyanophage are currently unknown. It has been hypothesized that lysogeny is a survival mechanism activated in response to resource limitation. Phosphate limitation is one potential control over both lytic and lysogenic viral replication. For example, it has been demonstrated that viral replication could be inhibited under phosphate-limited conditions in the marine coccolithophorid phytoplankter *Emiliania huxleyi* (2).

Besides being a factor possibly limiting lytic viral replication, phosphate amendment has been shown to enable prophage induction on occasion in natural populations of heterotrophic bacteria (38). In *Synechococcus*, nutrient availability, especially of phosphate, affects the kinetics of cyanophage infection and may be responsible for the switch from lysogeny to lytic viral production (40, 41). It has also been suggested that the high affinity and uptake rate for inorganic phosphate in *Synechococcus* explain its predominance in the phytoplankton (10, 15).

A recent study on viral interactions in the water column with *Synechococcus* and its close relative *Prochlorococcus* demonstrated decreasing cyanophage titers along a transect from coastal to oligotrophic waters (26). The researchers in this study suggested that decreasing levels of nutrients favored lysogeny rather than lytic infection.

Our hypothesis was that lysogeny in *Synechococcus* might be more prevalent under nutrient-limited conditions and that cyanophage induction could be stimulated by nutrient addition. Experiments were performed in oligotrophic and coastal environments and in culture to determine if nutrient availability affects the switch from lysogeny to lytic viral production.

MATERIALS AND METHODS

Locations and sampling. The initial group of samples was obtained as a part of a 13-month seasonal study of prophage induction in cyanobacteria from 1999 to 2000. Natural seawater samples were obtained bimonthly from the St. Petersburg Pier located on the Tampa Bay estuary (Fig. 1). An additional sampling occurred in Tampa Bay in July 2002.

Samples were also obtained from the Gulf of Mexico during a research cruise during July 2001 representing differing nutrient regimens associated with the Mississippi River plume as it entered the oligotrophic Gulf of Mexico (34). Figure 1 is a 7-day composite Sea-Viewing Wide Field-of-View Sensor image of the Mississippi River plume marked with the locations of the sampling stations. Cyanophage induction experiments were performed at stations 1, 3, 7, and 10 with nutrient amendment inductions performed at stations 3, 7, and 10.

***Synechococcus* counts.** *Synechococcus* counts were performed using epifluorescence microscopy at blue excitation as previously described (31).

Cyanophage counts. To differentiate the cyanophage from the total viral population, the most probable number (MPN) method was utilized (28). A 1- to 5-dilution series of the environmental or prophage induction treatment sample was prepared using 96-well microtiter plates (Costar-Corning, Inc.). A susceptible *Synechococcus* host was then freshly diluted 1:10 and placed in each well (either *Synechococcus* isolate WH 7803; our own isolate, GM 9901; or both). Control plates were prepared similarly using sterile natural seawater medium in the first column of wells. Three replicate treatment and control plates were prepared from each site. The plates were incubated until good growth of the host organism was evident (10 to 14 days). Wells were scored as positive for virus if lysis of the host organism was evident. Viral abundance was calculated for each plate by using an MPN program (9).

Prior to being used in the MPN assay, both *Synechococcus* isolates were tested with a dilution series of mitomycin C. Slight growth inhibition was observed at the highest experimental concentrations but not at the diluted levels used in the assay. No cyanophage prophage induction was observed in either isolate (data not shown).

Prophage induction. The samples for prophage induction were pretreated by the technique of viral reduction (36). Briefly, each sample was filtered through a 0.2-μm-pore-size filter to a volume of approximately 5 ml to remove most of the ambient viruses. Virus-free (0.02-μm-filtered) water prepared from the same sample was added, and the volume was reduced a second time. The retentate was then returned to its original volume by the addition of virus-free seawater. The reconstituted sample was then divided into aliquots and incubated with or without nutrient amendments. The nutrient amendments consisted of a 50 μM final concentration of ammonium (NH_4Cl), nitrate ($NaNO_3$), or urea or a 10 μM final concentration of phosphate (KH_2PO_4). All chemicals used were obtained from Sigma Chemical Co., St. Louis, Mo. The compounds were prepared using reagent grade water and sterilized by autoclaving before use. Treatment samples were also amended with the inducing agent mitomycin C at a concentration of 1 μg/ml both with and without the same nutrients as the control samples.

The final group of environmental samples was obtained from Tampa Bay. Although *Prochlorococcus* and *Synechococcus* have been demonstrated to be poor food items for common types of zooplankton (5), these samples were prefiltered (1-μm pore size) to prevent potential interference from grazing organisms.

Viral production. Cyanophage viral production was calculated by the dilution technique as previously described (37). Measurements were based on the MPN cyanophage numbers for both non-nutrient-amended and nutrient-amended samples.

Nutrient analysis. Ambient nutrient concentrations were measured at stations 1, 3, and 7 for both filtered and unfiltered water samples. All water samples were stored in 30-ml polycarbonate bottles at −20°C until analyzed. The analytical laboratory of the Virginia Institute of Technology performed the nutrient analyses.

Statistical analysis. Two types of statistical analysis were performed. First, treatment and control cyanophage and *Synechococcus* counts were evaluated by a paired *t* test between samples by using Minitab statistical software. Comparison of induction results and environmental parameters was also performed by linear regression and chi-square analysis, also using Minitab. Secondly, multivariate analyses of cyanophage induction parameters and measured environmental pa-

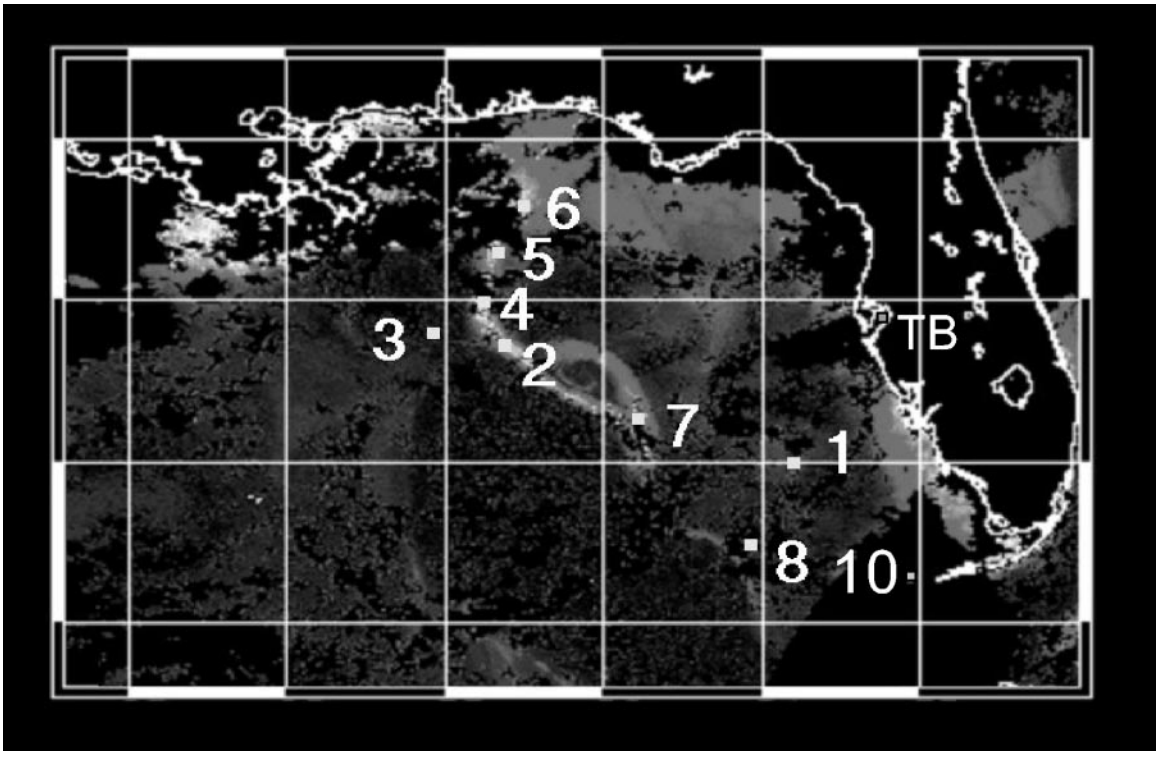

FIG. 1. Sea-Viewing Wide Field-of-View Sensor image of the Gulf of Mexico with sampling locations numbered. TB, Tampa Bay.

rameters were performed using Primer version 5.2.9 software (Primer-E Ltd., Plymouth Marine Laboratory, Plymouth, United Kingdom [http://www.primer-e.com]). Initially, the similarity matrices of both the cyanophage induction parameters and the environmental parameters constructed utilizing normalized Euclidian distances were compared to determine if a statistically significant relationship existed between the matrices utilizing the RELATE test (sample statistic ρ). The matrices were then optimized using both fourth-root and log transformations and compared by the BIOENV test to determine which environmental parameters best accounted for the observed distribution of cyanophage induction.

Prophage induction in *Synechococcus* cultures. Preliminary phosphate enrichment induction experiments were also performed with a known lysogenic *Synechococcus* isolate (strain GM 9914) in our culture collection. The isolate in log-phase growth was divided into four separate flasks and amended in a similar fashion to that in the cruise experiments. One flask received no amendment and served as control. One flask received 0.5 μg of mitomycin C ml^{-1}, one received a 10 μM phosphate amendment (KH_2PO_4), and the fourth flask received both phosphate and mitomycin C. Viral abundance for each treatment was monitored by SYBR gold staining (16).

RESULTS

To determine if elevated phosphate levels could stimulate or enable prophage induction in *Synechococcus*, phosphate-enriched treatments were added to the latter part of a 13-month seasonal study on lysogeny in *Synechococcus*. Of the nine phosphate-enrichment experiments performed, cyanophage induction was observed in only three (Fig. 2).

In the September sampling, the phosphate enrichments appeared to enable cyanophage induction. This effect was observed in the dark incubation with phosphate amendment in combination with the inducing agent mitomycin C and not with the phosphate enrichment alone or mitomycin C alone. In the July sampling, prophage induction was observed only in the phosphate-enriched mitomycin C sample. However, the increase in the level of cyanophage induction observed, measured as the percent change over control, was attributable to a decrease in the phosphate-enriched controls. This decrease was not statistically significant in these initial studies and was also not observed in the September positive induction. In addition, the levels of cyanophage in both the nutrient-enriched and nonenriched mitomycin C samples were similar. In the August positive-induction experiment, prophage induction occurred at a similar level in response to mitomycin C in both the presence and absence of phosphate amendment, indicating that phosphate did not stimulate or enable prophage induction.

To further investigate the effect of nutrients on lysogeny, cyanophage induction experiments were conducted during a research cruise along a transect of the Mississippi River plume (Fig. 1) during the summer of 2001. A variety of nutrient conditions were available for testing the hypothesis that nutrient status would affect the presence of lysogeny in *Synechococcus* populations and the response to nutrient stimulation.

With the exception of station 10, the cyanophage counts were approximately an order of magnitude less than the *Synechococcus* counts in both offshore and coastal environments (Fig. 3). At station 10, the cyanophage counts were higher than the *Synechococcus* counts, as well as being higher than the cyanophage counts at all other stations sampled.

Station 1 was an oligotrophic offshore site outside of the Mississippi plume. A large (1,463%) increase in cyanophage was observed at this station in response to mitomycin C treatment ($P < 0.0005$ [data not shown]). No nutrient amendments

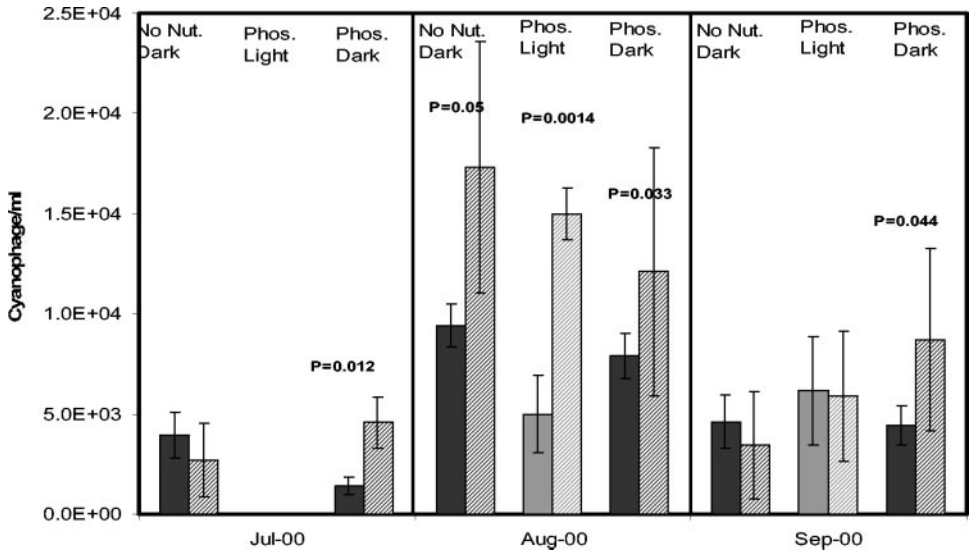

FIG. 2. The effect of phosphate enrichment on prophage induction during the latter part of a seasonal study in Tampa Bay in 2000. Solid bars indicate control samples; crosshatched bars indicate mitomycin C treatment. Experimental conditions included non-nutrient-amended dark incubations (No Nut. Dark) and phosphate-amended samples incubated both in the light (Phos. Light) and in the dark (Phos. Dark). Numbers above the bars indicate levels of statistical significance.

were performed at this station. The inorganic nitrogen/phosphate (N/P) ratio of 1.25 suggested that nitrogen limitation may have occurred (Table 1). In addition to having the lowest N/P ratio, this station had the lowest overall abundance of *Synechococcus* and cyanophage.

Station 3 was located along the edge of the proximal plume of the Mississippi River, with an inorganic N/P ratio of 8.94. The increase in cyanophage was not significant in the non-nutrient-amended mitomycin C-treated samples compared to the control samples (baseline cyanophage induction). However, significant inductions were observed at the 90% confidence interval in response to nitrate and ammonium enrichments and at a >95% confidence interval in response to phosphate enrichment (Fig. 4). The numbers of cyanophage produced in response to mitomycin C induction were very similar in both the nutrient-enriched and nonenriched treatment samples. However, decreases in the cyanophage titer in the nutrient-enriched controls resulted in a calculated significant prophage induction in the nitrate- and phosphate-amended samples.

Station 7 was located within the distal Mississippi River plume. The inorganic N/P ratio of 1.99 suggested that nitrogen limitation may have occurred at this station. At this site, there was a statistically significant ($P < 0.05$) induction in the baseline non-nutrient-amended sample as well as a statistically significant response to nutrient enrichment. Based on percent change estimations, the nutrient-amended samples demonstrated a higher level of cyanophage induction. Similar to station 3, this was due to a decrease in the cyanophage titer in all of the nutrient-amended controls (Fig. 5). In contrast to station 3, the decrease in the level of cyanophage in the nutrient-amended controls in comparison to that in the nonamended control was statistically significant ($P < 0.05$). No significant inductions were detected at station 10.

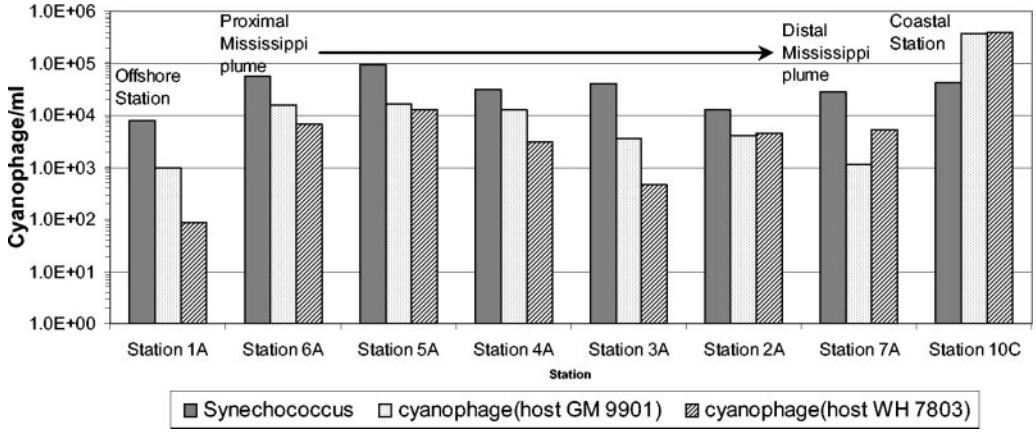

FIG. 3. Ambient level of *Synechococcus* and cyanophage at all cruise stations sampled in 2001 (see Fig. 1 for station locations).

TABLE 1. Station parameters from a Gulf of Mexico cruise

Station	Water temp (°C)	Salinity	NH_3 (μmol liter^{-1})	$NO_2 + NO_2$ (μmol liter^{-1})	PO_4 (μmol liter^{-1})	N/P ratio
1	29.3	35.12	0.321	0.05	0.297	1.25
3	28.9	34.2	0.178	2.271	0.274	8.90
7	29.7	33.5	0.271	0.343	0.277	1.99
10	30.5	40	Not done	Not done	Not done	Not done

Additional cyanophage induction experiments were performed with Tampa Bay water samples incubated with the nutrient amendments for 4 h prior to the addition of mitomycin C for two reasons. The first reason was to determine the effects of nutrient addition on growth of the host organisms and on viral production rates in nutrient-amended controls (Fig. 6). The second reason was to help prevent any potential interaction between the added nutrient and the mitomycin C.

Growth of the *Synechococcus* was affected by the nutrient amendments (Fig. 6). With all three of the nutrient additions, initial growth of the host was slowed in comparison to the control at 4 h, with all counts similarly decreased by 24 h. The *Synechococcus* cells in the nutrient-amended samples were also more strongly affected by the mitomycin C treatment than those in the non-nutrient-amended sample (Fig. 6). This decrease in cell abundance in response to mitomycin C in the nutrient-amended samples at 24 h was significant at the 94% confidence interval with *P* values of approximately 0.06 for all three nutrient amendments.

Four-hour viral production rates were also decreased in the nutrient-amended samples in the additional Tampa Bay experiment. By utilizing host organism GM 9901 to enumerate cyanophage abundance, the baseline cyanophage viral production rate was 2.2×10^5 cyanophages/h. Addition of nitrate, ammonium, and phosphate decreased the viral production rates to 9.1×10^3, 9.4×10^3, and 7.6×10^3 cyanophages/h respectively. By utilizing host organism WH 7803 for enumeration, the baseline level of viral production was estimated to be 2.9×10^5 cyanophages/h. The same nutrient additions led to decreased production rates of 1.3×10^4, 3.5×10^3, and 4.6×10^3 cyanophages/h, respectively. This decrease in cyanophage production observed with the addition of inorganic nutrients is consistent with the observations of decreased cyanophage abundance in nutrient-amended controls in the offshore waters (stations 3 and 7) described above.

In the additional Tampa Bay experiment, a statistically significant level of cyanophage induction ($P < 0.05$) was detected in the non-nutrient-amended sample utilizing both *Synechococcus* host organisms (Fig. 7). Both host organisms also demonstrated a significant increase in the level of cyanophage in the phosphate-amended non-mitomycin C sample. Based on our criterion for nutrient-stimulated induction, these results indicated that the phosphate amendment might have stimulated lytic cyanophage production in this experiment but not prophage induction.

The WH 7803 host also enabled detection of a significant increase in cyanophage in the ammonium-amended sample compared to that in the nonamended control. However, the ammonium plus mitomycin C sample was significantly higher than the nutrient-amended sample alone. This result indicated that the ammonium enrichment might have stimulated lytic viral production while still enabling detection of mitomycin C-stimulated prophage induction (Fig. 7).

In the experiment performed with the lysogenic *Synechococcus* strain GM 9914, a similar decrease in level of viruses was observed in response to the phosphate amendment (Fig. 8). A statistically significant prophage induction was observed in response to the mitomycin C treatment. There was also a significant decrease in the level of virus in the phosphate-amended sample compared to that in the negative control. In addition, prophage induction appeared to be suppressed in the phosphate plus mitomycin C-treated sample (Fig. 8).

DISCUSSION

Prior work has shown that mitomycin C prophage induction has occurred variably over space and time in natural populations of both heterotrophic bacteria and picocyanobacteria (14, 19, 38). The goal of our study was to determine if the detection of lysogens by this assay might underestimate the occurrence of lysogeny because of nutrient limitation. Cells may lack sufficient nitrogen or phosphorus to enable induction to occur in the presence of this artificial inducing agent. Only one of the Tampa Bay samples (Fig. 2) demonstrated greater induction of the prophage with nutrient addition (phosphate) plus mitomycin C rather than with mitomycin C alone.

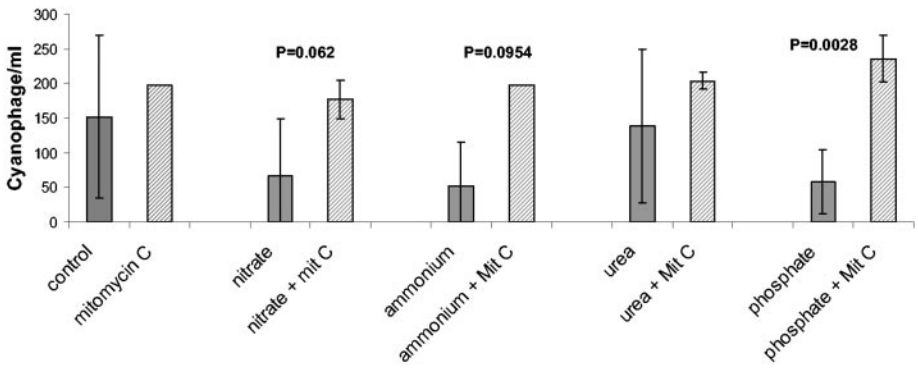

FIG. 4. Cyanophage induction experiments from cruise station 3. Solid bars indicate control samples; crosshatched bars indicate mitomycin C treatment. Error bars indicate standard deviations.

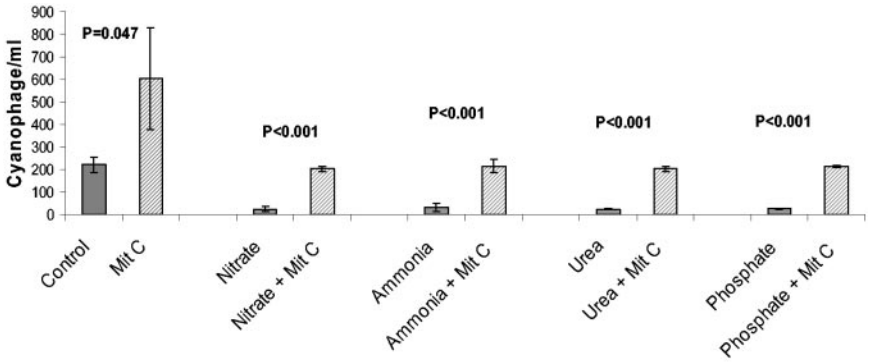

FIG. 5. Cyanophage induction experiments from cruise station 7. Solid bars indicate control samples; crosshatched bars indicate mitomycin C treatment. Error bars indicate standard deviations.

At offshore stations 1 and 7, both areas with low N/P ratios, mitomycin C stimulated prophage induction without the addition of any nutrients. It may be that nitrogen-limited oceanic environments may favor the occurrence of lysogeny. At offshore stations 3 and 7 and coastal station 10 and in Tampa Bay samples and culture samples, nutrient addition resulted in a decrease in viral abundance in control (non-mitomycin C-treated) samples. These results suggested that nutrient addition has a general inhibitory effect on lytic viral production and/or spontaneous induction.

At station 10, there was no detectable cyanophage induction in response to either the nutrients or mitomycin C. These findings suggested that the cyanophage was already induced by natural conditions at this site, leading to the high cyanophage abundances. Alternatively, there may have been no lysogenic *Synechococcus* at this site. There was also a significant decrease in viruses in the nutrient-amended controls at this station.

Unlike the environmental isolates, the phosphate-amended plus mitomycin C-treated lysogenic marine *Synechococcus* isolate demonstrated an inhibition of prophage induction. It is possible that the addition of nutrients and mitomycin C simultaneously may have led to some synergistic or antagonistic effects between the compounds. However, the differing reactions observed in cells in differing physiological condition (i.e., nutrient-limited environmental cells versus nutrient-replete cultures) argue against this. Further experimentation will be needed to determine this conclusively.

Although treatment with mitomycin C is an artificial way of inducing cyanophage, it does allow the experimental detection of inducible prophage and has been the agent of choice for this purpose for many studies. Our criterion for nutrient limitation of the process of cyanophage induction was the observation of a significant increase in samples amended with nutrient plus mitomycin C over the samples amended with the nutrient alone, in conjunction with no cyanophage induction in samples amended with mitomycin C alone. Based on our criteria, it appeared that nutrient amendment sometimes inhibits lytic viral production while still allowing mitomycin C-stimulated induction, leading to a relative increase in the level of prophage induction. However, prophage induction stimulated by the nutrient addition alone only occurred once in this study (Tampa Bay seasonal sampling from September).

It has been hypothesized that nutrient amendments alone may "turn the lysogenic switch," leading to prophage induction with lytic production of cyanophage. This would have led to the observation of a significant increase in the level of cyanophage in the nutrient-amended samples in comparison to controls. In this case, if the nutrients alone were stimulating prophage induction, a comparable, significant increase in viruses would be expected in the mitomycin C-treated sample as well, which was not observed. It is possible this was caused by some synergistic effect between phosphate and mitomycin C. However, this seems unlikely since, during the August seasonal experiment and cruise experiments, prophage induction did occur and the nutrient- and non-nutrient-enriched mitomycin C treatments were statistically indistinguishable.

Another comparable set of experiments on heterotrophic bacterial populations in the Gulf of Mexico led to some similar conclusions (39). Although the ambient heterotrophic and autotrophic bacterial populations responded differently to the

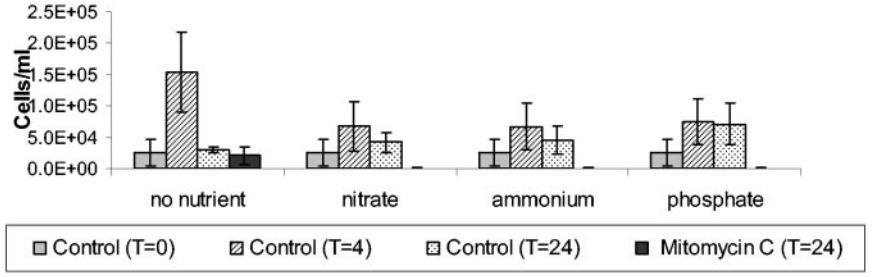

FIG. 6. *Synechococcus* counts in control and nutrient-amended samples from the Tampa Bay induction experiment at 4 and 24 h and in mitomycin C-treated samples at 24 h. Error bars indicate standard deviations.

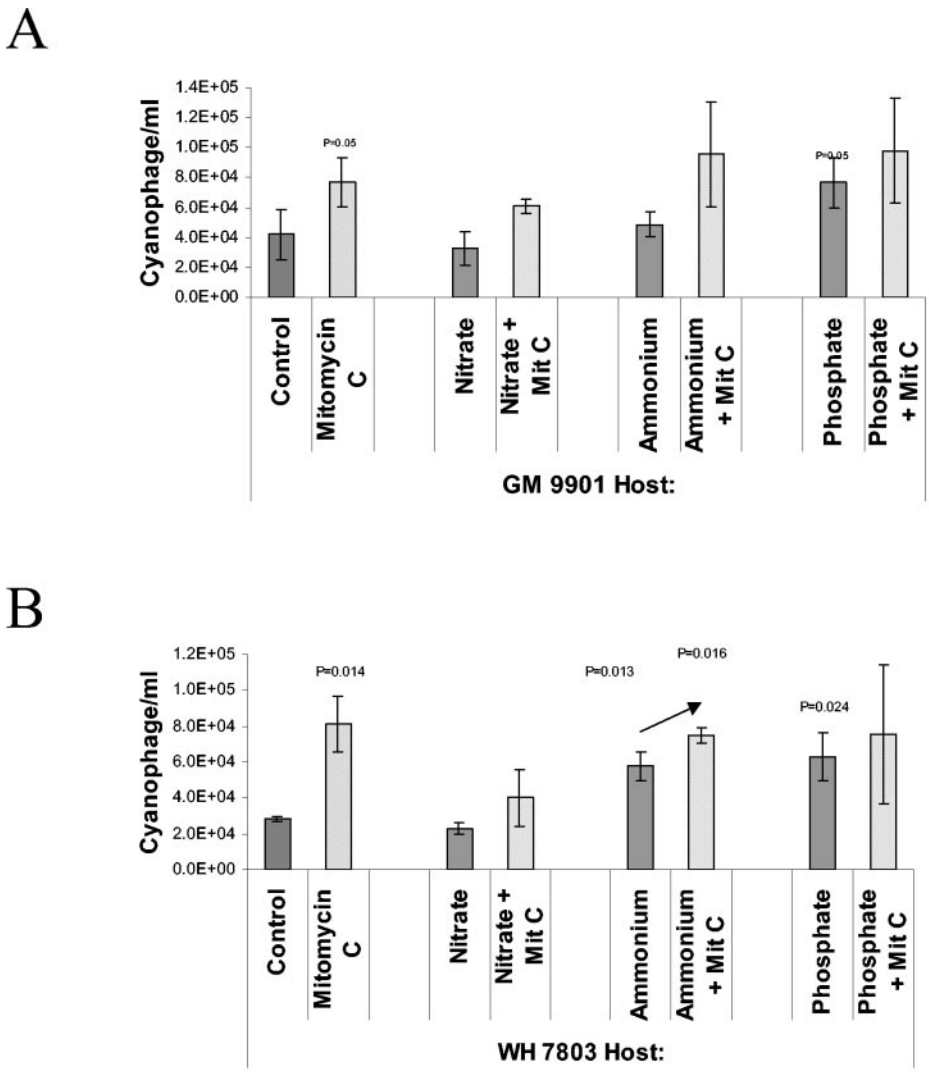

FIG. 7. (A) Tampa Bay induction experiment with change in the level of cyanophage detected utilizing *Synechococcus* host GM 9901. The experiment was performed concurrently with the experiment described for panel B. Solid bars indicate control samples; crosshatched bars indicate mitomycin C treatment. Numbers above the bars indicate the levels of statistical significance with error bars indicating standard deviations. (B) Tampa Bay induction experiment utilizing *Synechococcus* host WH 7803 to detect the level of cyanophage. Solid bars indicate control samples; crosshatched bars indicate mitomycin C treatment. In the ammonium-enriched sample, the arrow indicates a statistically significant increase ($P = 0.016$) in the sample containing ammonium and mitomycin C in comparison to that in the sample amended with mitomycin C alone.

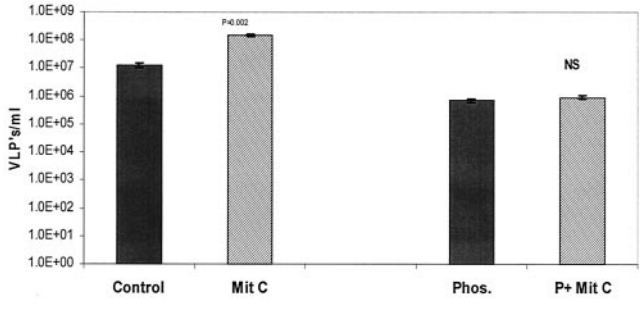

FIG. 8. Nutrient enrichment induction experiments with a lysogenic *Synechococcus* isolate. Solid bars indicate control samples; crosshatched bars indicate mitomycin C-treated samples. Note the logarithmic scale.

addition of nutrient supplements, increases in lytic viral production were observed rather than stimulation of prophage induction. Only one experiment using a 0.1 μM concentration of phosphate demonstrated clear stimulation of prophage induction. This led to the parallel conclusion that, for the heterotrophic bacterial populations examined, the process of prophage induction is rarely nutrient limited.

Relative nutrient limitation in the phytoplankton populations was estimated utilizing the N/P ratio since the phosphate concentration was similar at all stations. It was observed that lysogeny could be detected in offshore populations that were nitrogen limited as determined by the inorganic N/P ratio. This relationship was not observed for the estuarine samples. It is possible that inorganic nutrient levels alone may give an incomplete indication of the actual nutritional status of the population. The completed sequence of *Synechococcus* WH 8102

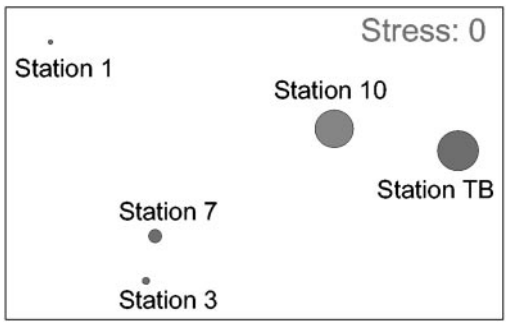

FIG. 9. Multidimensional scaling plot of the level of cyanophage induction from all experiments performed. The circles denote the ambient level of cyanophage.

has indicated the presence of genes for the utilization of organic nitrogen sources as well as genes for the utilization of alternate sources of phosphorus (21). If these genes are common in *Synechococcus*, assessment of relative nutrient limitation in natural populations will be more difficult, especially in more productive estuarine environments.

The *Synechococcus* abundances at most of the cruise stations were higher than their corresponding cyanophage. This is most likely an underreporting of the true cyanophage abundance as an artifact of the MPN method. A comparison of the MPN method of enumeration of viruses with both fluorescence microscopy and plaque assay methods has demonstrated that the MPN method gives an overall viral abundance lower than microscopy and higher than plaque assay due to the detection of host-specific and infective viruses only. However, all three methods demonstrated a similar level of precision (8).

The MPN method is very useful in the evaluation of environmental samples because it can separate the cyanophage from the ambient viral community. Since the host range of cyanophages varies, the host selection will alter the estimated cyanophage titer (13, 28). It has been demonstrated in previous experiments that using a phycoerythrin-containing host organism that was isolated from an offshore environment and has been maintained in laboratory culture free from virus pressure for several years will yield the highest cyanophage counts (28, 32). We used the isolate WH 7803, which meets these criteria along with being identified as an organism highly susceptible to a wide variety of cyanophages. In addition, we utilized our own isolate, GM 9901, which is a phycoerythrin-containing marine 9901 isolate closely related to WH 7803 by rbcL phylogeny (data not shown). This isolate has consistently revealed a higher titer of cyanophage than WH 7803 ($P = 0.002$).

Multivariate statistical analysis is capable of ascertaining significant correlations based on combined factors, which might not be evident using either linear or multiple linear regressions (6). This method constructs a three-dimensional distribution of all prophage induction variables. The multidimensional pattern of the distribution of cyanophage induction can be depicted in a multidimensional scaling diagram (MDS plot). The clear separation between the oligotrophic, coastal, and eutrophic sampling sites can be readily discerned (Fig. 9). The distribution of cyanophage induction can then be compared to all measured environmental parameters measured, both separately and in combination with each other to determine correlations between cyanophage induction and the environmental variables.

Multivariate analysis indicated that there was a significant correlation between cyanophage induction and measured environmental variables ($\rho = 0.818$, $P = 0.007$). Further optimization of the analysis by utilizing transformed data indicated that the level of cyanophage induction, measured by both the parameters of cyanophage per milliliter and percent change, were most closely and negatively correlated with the three combined parameters of temperature, *Synechococcus* abundance, and cyanophage abundance ($\rho = 0.867$). The level of cyanophage induction was higher under the combined conditions of lower temperature, lower ambient *Synechococcus* abundance, and lower ambient level of cyanophage. There was insufficient data to analyze the contribution of ambient nutrient level to the observed distribution of cyanophage induction.

Reanalysis of the data from a yearlong seasonal study of cyanophage induction (14) utilizing the multivariate RELATE and BIOENV tests provided additional support for these conclusions. This analysis demonstrated that there was a significant relationship between the level of cyanophage induction and at least some of the measured environmental parameters ($P = 0.01$). The best observed correlation was an inverse relationship between the level of cyanophage induction and ambient level of cyanophage ($\rho = 0.812$). Excellent correlation was also obtained between the level of prophage induction and the combined factors of ambient level of cyanophage and ambient level of *Synechococcus* ($\rho = 0.780$).

A recent study of the prevalence of lysogeny in heterotrophic populations from surface, mesopelagic, and deep waters of the Mediterranean and Baltic Seas showed that the prevalence of lysogeny was inversely correlated with host abundance, followed by viral abundance and bacterial production (35). We found a similar relationship between the occurrence of lysogeny and primary productivity during the seasonal study of lysogeny in Tampa Bay (14).

The Gulf of Mexico cruise experiments also demonstrated an inverse correlation between primary productivity and level of cyanophage induction. However, the number of stations with data for comparison provided too small a sample to determine statistical significance at a high confidence interval (Pearson correlation, $r = -0.9525$, $P = 0.14$, $n = 3$).

These experiments have indicated that nutrient amendment alone is rarely sufficient to stimulate prophage induction in *Synechococcus.* In both coastal and oligotrophic environments, it appears to be the ambient level of cyanophage and *Synechococcus* hosts, possibly in combination with temperature and level of productivity, that determines the prevalence of lysogeny and the level of prophage induction in natural populations of *Synechococcus.*

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