Potential Significance of Lysogeny to Bacteriophage Production and Bacterial Mortality in Coastal Waters of the Gulf of Mexico

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The potential effect that induction of lysogenic bacteria has on bacteriophage production and bacterial mortality in coastal waters was investigated, and we present estimates for the percentage of lysogenic cells in a natural aquatic bacterial community. Various concentrations of mitomycin C and exposure times to UV C radiation (UV-C) (wavelength of 254 nm) were used to induce the lytic cycle in lysogenic cells of natural communities of marine bacteria. UV-C treatment occasionally resulted in phage production, but phage production induced by UV-C was always less than that caused by the addition of mitomycin C. There was no evidence that high growth rates of bacteria resulted in lysogenic phage production. The burst size of cells induced by mitomycin C was determined by transmission electron microscopy and ranged from 11 to 45. Dividing the induced phage production by the burst size provided an estimate of the number of lysogenic bacterial cells, which ranged from 0.07 to 4.4% (average, 1.5%) of the total bacterial population. The percentages of lysogenic bacteria that were induced by mitomycin C were similar for samples collected nearshore from the pier of the Marine Science Institute (chlorophyll *a*, 1.6 to 2.9 $\mu\text{g liter}^{-1}$) and in relatively oligotrophic water (chlorophyll *a*, 0.2 to 0.9 $\mu\text{g liter}^{-1}$) collected 25 to 100 km offshore. By using a steady-state model, if all lysogenic bacteria were induced simultaneously, 0.14 to 8.8% (average, 3.0%) of the total bacterial mortality would result from induction of lysogenic cells. If mitomycin C induces all or the majority of lysogenized cells, our results imply that lysogenic phage production is generally not an important source of phage production or bacterial mortality in the coastal waters of the western Gulf of Mexico.

The earliest reports of bacteriophage activity in marine ecosystems date back to the first quarter of this century (36), although the existence of indigenous marine bacteriophages was not confirmed until 1955 (24). More than two decades later, Torrella and Morita (31) provided minimum estimates of viral abundances of $>10^4$ particles ml^{-1} . It is now known that viral abundances typically exceed those of bacteria and routinely occur at $>10^7$ particles ml^{-1} (2, 21). Currently, bacteriophages are considered to be dynamic partners in microbial food webs (3, 4, 6), and it is thought that, on average, approximately 20% of the standing stock of bacteria are lysed by phages on a daily basis (27).

There are two major pathways of phage replication that can occur when a phage infects a suitable host (14). One is the lytic cycle, in which the phage genome replicates immediately after infection and the newly formed viral progeny are released as the host cell bursts. The other is the lysogenic cycle, in which the genome of the phage is carried by the host cell from generation to generation in a noninfectious form, until the lytic cycle is induced by factors such as UV radiation or vigorous growth of the host. Phages that can establish lysogeny with their hosts are called temperate, whereas those that cannot are termed virulent.

Two major hypotheses have been put forward to explain the advantages of lysogeny (25). The first is that since lysogenized cells are immune to infection by the same phage strain, they have a competitive advantage over nonlysogenized cells, which can be infected and lysed. The second explanation is that

lysogeny is a potential survival strategy to maintain phage populations when host abundance is too low for populations to be maintained by lytic infection. Since modest manipulations of seawater have been reported to cause viral production, it has been suggested that lysogeny predominates in planktonic systems (4, 30). Moebus (17) found that about 10% of the phages isolated from marine systems were temperate, whereas Jiang and Paul (10) showed that 43% of the bacteria isolated from different marine habitats were induced with mitomycin C. However, Wilcox and Fuhrman (35) found no evidence for lysogenic bacteriophage production in natural bacterial communities from coastal seawater. The production of temperate phages is dependent on the number of lysogenic bacteria and the presence of an inducing agent, whereas virulent phage production depends on the encounter rate between phages and host cells. Thus, the effects that phages can have on mortality, nutrient flow, and species diversity in microbial food webs are influenced by the mode of viral replication.

Typically, chemical means or UV C radiation (UV-C) is used to damage DNA or inhibit DNA replication; this activates DNA repair mechanisms, including the RecA protein which cleaves a repressor and induces prophages to enter the lytic cycle. One of the most effective and widely used inducing agents is mitomycin C (1). It has been used by a number of investigators to examine lysogeny in marine viral communities and isolates (10, 11, 29). Although some prophages cannot be induced by mitomycin C or UV-C (5), there is no evidence that such lysogenic associations occur in high relative abundance in the sea or elsewhere. Hence, in the absence of data which indicate otherwise, it is reasonable to assume that mitomycin C and UV-C are also effective inducing agents for marine lysogens.

The approach used to investigate lysogeny in marine bacteria in this study was to expose natural marine microbial communities to mitomycin C or UV-C and to quantify the number

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of viruses produced by lysogenized cells. This provided an estimate of the maximum potential viral production by lysogens. For each experiment we also estimated the number of viral particles produced per lytic event (burst size). Dividing the total number of viruses induced by the burst size provided an estimate of the number of lysogens in the microbial community. If the assumption is correct that mitomycin C can induce most lysogenic cells, the percentage of lysogenic bacteria is typically low and lysogenic phage production is unlikely to explain high abundances of infectious viruses in surface waters of the Gulf of Mexico.

MATERIALS AND METHODS

Study sites and sampling. Seawater for incubations was collected from surface water at the pier of the Marine Science Institute (station A: 27°50'N, 97°02'W) and from water below the pycnocline at stations about 25 km (station B: 27°39'N, 96°55'W), 50 km (station C: 27°32'N, 96°45'W), and 100 km (station D: 26°47'N, 96°23'W) offshore in the Gulf of Mexico. At the pier, seawater was collected before sunrise, or in one instance (3 January 1995) during the afternoon of a heavily overcast day, in order to avoid exposure of seawater to strong visible or UV light. Samples from the pier were collected and transported to the lab in a sterile 10-liter polypropylene container that was prerinsed with seawater prior to sample collection. Samples at the offshore stations were collected in Go-Flo bottles mounted on a rosette equipped with a Sea-Bird CTD (model SBE 9 plus). Duplicate subsamples for the enumeration of viruses and bacteria, and the determination of burst size, were preserved with glutaraldehyde (1% final concentration) immediately after collection of the seawater. Samples were also taken for the determination of chlorophyll *a* (Chl *a*) concentrations. Nine experiments were performed between 11 November 1994 and 28 June 1995.

Approach. There was a potential for the accuracy of the results from these experiments to be compromised by viral production resulting from lytic viral infection. Consequently, it was necessary to reduce the concentration of viral particles in the seawater, so that contact rates between viruses and host cells were greatly reduced and the potential for new infections to occur was minimized. This was accomplished by rinsing the natural bacterial community with seawater that was free of infectious viruses, and, in several experiments, by dilution of samples with heat-treated seawater or an ultrafiltrate produced from seawater. Using the model presented by Murray and Jackson (18) for calculating encounter rates between viruses and bacteria, and assuming a diameter of a typical bacterial cell of 0.5 μm , we estimated that subsequent to the reduction of viral abundances by washing and dilution, <0.6% of the bacterial community would be contacted per hour by a virus. Since the diversity of bacteria and viruses in natural communities is probably high, only a small proportion of these encounters would cause infection. Thus, by reducing the encounter rates, we basically eliminated lytic phage production in the experiments. Reducing viral concentrations also allowed for more-accurate estimates of lysogenic viral production by reducing the background concentration of viral particles. Moreover, dilution of the samples with heat-treated seawater or an ultrafiltrate minimized the possibility that the experiments were compromised by nutrient limitation of the microbial community.

It is possible that the concentration and subsequent dilution of the natural microbial community could affect growth rate. As we were interested only in the proportion of the bacterial community that was lysogenized, however, it was not important whether growth was anomalous or typical. Rather, it was important that cells which were lysogenized could be induced. A potential problem would exist if these manipulations resulted in the induction of lysogens. Consequently, we counted viral particles and bacteria at each step of the procedure to determine if lysogens were being induced by the procedure.

Incubation media. The incubation medium for the first experiment was made from a virus-free ultrafiltrate produced by filtering seawater through an ultrafiltration cartridge with a 30,000-molecular-weight cutoff, as previously described (28). As the ultrafiltrate had been collected several weeks before the experiment, immediately before use it was refiltered through a 0.2- μm -pore-size filter to remove any contaminating bacteria. In this experiment bacterial abundances increased only slightly during a 72-h incubation, probably as a result of nutrient depletion due to bacterial growth in the ultrafiltrate during storage.

Media for the other experiments performed in winter were made from seawater that was collected from the pier, filtered through 20- μm -pore-size Nitex screening, and sterilized by heating in a microwave or autoclave. Seawater (1.5 liters) was microwaved for 30 min, in 5-min intervals. Between intervals the seawater was chilled to about 65°C on ice in order to keep the maximum temperature below 100°C. After heat sterilization the media were filtered through 1.0- and 0.2- μm -pore-size polycarbonate filters and stored at 4°C for 1 to 2 days prior to experiments. The pH of the seawater was not affected by these treatments. In the experiments performed in June 1995, the ultrafiltrate that was produced from seawater immediately before the onset of the experiments was used as the medium. Duplicate subsamples for the determination of viral and bacterial abundances in the media were collected immediately before the start of the experiments and preserved in glutaraldehyde (1% final concentration).

Incubations. Seawater samples for the incubations were processed immediately after collection. Between 300 and 1,500 ml of seawater was filtered through a 1.0- μm -pore-size polycarbonate filter, and the bacteria in the filtrate were concentrated about 10-fold by using a 0.2- μm -pore-size polycarbonate filter. The bacteria were rinsed by adding 50 ml of virus-free medium to the concentrated bacteria and refiltering this volume. The washed concentrate was made up to the original volume (between 300 and 1,500 ml) with medium and transferred to beakers. The filters with which the bacteria were concentrated were washed in these samples to release attached bacteria. This procedure decreased the concentration of viruses by about 10-fold, while the bacteria were maintained near their original concentration. In the first two experiments, this inoculum was used for the incubations. Subsamples for bacterial and viral counts were immediately preserved after filtration through the 1.0- and 0.2- μm -pore-size filters and after the 0.2- μm -pore-size filters were rinsed in the samples. In the subsequent experiments the bacterial and viral concentrations were further reduced by 90% through dilution of the samples with autoclaved seawater or the ultrafiltrate. Dilution of the samples decreased contact rates between viruses and bacteria and thereby reduced production of viruses via the lytic cycle. Thus, the viral production that was observed after the induction of lysogenized cells with UV-C or mitomycin C could be attributed to lysogenic phage production. Viral production in the treated samples was compared with that of samples treated in the same manner but not exposed to inducing agents. Since nutrient limitation potentially could decrease induction of lysogenized bacteria, growth rates of bacteria were enhanced in some experiments by using microwaved or autoclaved seawater as the medium.

Duplicate samples (50 to 200 ml) prepared as outlined above were incubated in sterile petri dishes or in acid-rinsed 250-ml Erlenmeyer flasks. Short-term exposure of 50-ml samples to 2,000 to 300,000 $\mu\text{J cm}^{-2}$ of UV-C at a wavelength of 254 nm was carried out in open petri dishes by using a UV cross-linker (FisherBiotech FB-UVXL). For the chemical treatments, mitomycin C (Sigma Chemical Co. catalog no. M-0503; 1 mg ml⁻¹ deionized water) was added to the incubations at final concentrations of 0.2, 0.5, 1.0, and 2.0 $\mu\text{g ml}^{-1}$. In the first two experiments it was determined that the maximum number of viruses was produced at a mitomycin C concentration of 1.0 $\mu\text{g ml}^{-1}$; hence, this concentration was used in the subsequent experiments. Untreated duplicate incubations served as controls.

Mitomycin C and UV-C are commonly used to induce lysogenic cells; however, vigorous growth can also cause lysogenic phage production (7). Thus, in three experiments, two replicates of the control incubations were treated with mitomycin C when bacteria entered exponential growth. If all lysogenized cells were induced at high growth rates, no further phage production would occur when these cultures were treated with mitomycin C.

Incubations were kept at in situ temperature ($\pm 1.5^\circ\text{C}$) except for the incubation on 3 January 1995, when the temperature increased from 13.5 to 19.5°C following dilution of the sample with the autoclaved medium. Subsamples for the enumeration of viruses and bacteria and the determination of the burst size were removed periodically and preserved with glutaraldehyde (1% final concentration).

Bacterial and viral counts and Chl *a* concentrations. Glutaraldehyde-preserved samples were kept at 4°C in the dark and processed within 3 days. Bacteria and viruses were stained with 4',6-diamidino-2-phenylindole (DAPI; final concentration, 5 $\mu\text{g ml}^{-1}$) and enumerated by epifluorescence microscopy, with a slight modification of the protocols described by Turley (32) and Suttle (26). Samples for bacteria and viruses were stained for 30 min, and up to 4.75 ml was filtered onto 0.02- μm -pore-size Anodisc filters (Whatman). In the first experiment, additional samples were stained for 5 min and filtered onto 0.2- μm -pore-size black polycarbonate filters (Poretics). There was no difference between estimates of bacterial abundance obtained with the Anodisc and polycarbonate filters (data not shown). In addition, estimates of viral abundance obtained by Yo-Pro (8) or DAPI staining were not significantly different (data not shown). Therefore, bacteria and viruses were enumerated on the same 0.02- μm -pore-size filters on which the subsamples had been filtered. Chl *a* concentrations were determined fluorometrically (19).

Determination of burst size. Bacteria were collected quantitatively onto Formvar-coated, 400-mesh electron microscope grids by centrifugation in a swinging-bucket SW40 rotor (70,950 $\times g$ for 20 min), stained for 30 s with 1% uranyl acetate, and rinsed three times with deionized distilled water. The chosen time and speed of centrifugation avoid disruption of infected bacteria, and as few viruses are pelleted, phages within bacteria are easily distinguishable. The viral burst size was estimated for each sample by counting the number of viral particles in a minimum of 15 visibly infected bacteria as described previously (33). A minimum of 7 phages was observed in a visibly infected cell. Burst size was estimated as the average number of viral particles in all visibly infected bacteria (minimum burst size) or as the average number of viruses in cells completely filled with viral particles (maximum burst size).

RESULTS

The water temperatures during the winter sampling period fell within 13.7 to 22.1°C but were >28°C during the June sampling dates (Table 1). The Chl *a* concentrations were con-

TABLE 1. Water temperature, Chl *a* concentrations, bacterial and viral abundances, and viral burst sizes at the various sampling sites

Date of sampling	Station ^a	Temp (°C)	Chl <i>a</i> concn ($\mu\text{g liter}^{-1}$)	Abundance ^b		Burst size ^{b,c}	
				Bacteria (10^6 ml^{-1})	Viruses (10^7 ml^{-1})	Minimum	Maximum
11 Nov 94	A	22.0	1.6	3.7 ± 0.30	4.9 ± 0.63	25 ± 3.2	33 ± 3.4
26 Nov 94	A	22.1	2.8	3.9 ± 0.30	3.0 ± 0.27	22 ± 1.9	31 ± 0.8
06 Dec 94	A	21.5	2.7	3.1 ± 0.40	1.5 ± 0.20	22 ± 3.1	29 ± 2.7
19 Dec 94	C	21.5	0.2	1.0 ± 0.09	0.4 ± 0.05	34 ± 4.5	54 ± 4.2
03 Jan 95	A	13.7	2.9	1.8 ± 0.09	0.4 ± 0.04	15 ± 2.3	26 ± 3.1
17 Jan 95	C	19.0	0.3	1.7 ± 0.09	0.2 ± 0.03	17 ± 1.7	29 ± 2.7
17 Jan 95	B	17.9	0.9	1.9 ± 0.19	0.4 ± 0.07	16 ± 0.9	23 ± 1.9
26 Jun 95	D	28.4	0.2	1.8 ± 0.10	0.5 ± 0.04	29 ± 8.4	40 ± 9.5
28 Jun 95	C	28.8	0.2	2.5 ± 0.19	0.7 ± 0.04	34 ± 7.8	47 ± 5.6

^a Stations: A, pier; B, 25 km offshore; C, 50 km offshore; D, 100 km offshore.

^b Values are expressed as the means $\pm$ standard deviations of duplicate samples.

^c Burst size estimated from the number of viral particles seen in all visibly infected cells (minimum) or only from the number of viruses in cells that were observed to be completely filled with viral particles (maximum).

sistently higher (1.6 to $2.9 \mu\text{g liter}^{-1}$) at the pier than at the more offshore stations (0.2 to $0.9 \mu\text{g liter}^{-1}$). Bacterial and viral abundances ranged from 1.0×10^6 to $3.9 \times 10^6 \text{ ml}^{-1}$ and viral numbers from 0.2×10^7 to $4.9 \times 10^7 \text{ ml}^{-1}$ (Table 1).

Bacterial and viral abundances in the microwaved or autoclaved media were always less than 5% of those at the start of the experiments (data not shown), and those that remained were presumably not viable. Across all experiments there was no significant difference in the abundance of bacteria following filtration through $1.0\text{-}\mu\text{m}$ -pore-size filters and the concentration procedure (paired *t* test, two tailed; $P > 0.05$). However, for individual sampling dates, bacterial abundance was estimated to decrease between 12.5 and 41.8% (average, $24.8\% \pm 11.83\%$) (Fig. 1), although the statistical significance of these decreases could not be determined because only duplicate determinations were made. On average, viral abundance decreased following filtration through 1.0- and $0.2\text{-}\mu\text{m}$ -pore-size filters by $10.1\% \pm 15.11\%$ (Fig. 1), although this decrease was not statistically significant (paired *t* test, two tailed; $P > 0.05$).

Growth of bacteria. In the first experiment bacterial abundance in the controls increased only slightly for 72 h. Since this might have resulted from nutrient depletion due to bacterial growth in the ultrafiltrate during storage, we used microwaved or autoclaved seawater or a fresh ultrafiltrate as the medium in the subsequent experiments. This medium supported substantial increases in bacterial abundance (Fig. 2 and 3), which plateaued at about 3×10^6 to $4 \times 10^6 \text{ cells ml}^{-1}$ in several experiments; decreases in bacterial abundances were not observed.

In incubations with mitomycin C treatment, bacterial growth ceased or was strongly reduced (Fig. 2 and 3). In contrast, UV-C treatments either had no effect or caused a delay in bacterial growth, with the response not consistently related to dose. However, at the highest UV-C dose used in this study ($300,000 \mu\text{J cm}^{-2}$), bacterial abundance was only about 10% of that in the control incubation at 4 days after exposure.

Viral dynamics and mitomycin C treatment. Typically, viral abundances remained comparatively stable in the controls during the first 10 to 24 h but increased after treatment with $1.0 \mu\text{g}$ of mitomycin C ml^{-1} (Fig. 2 and 3). The difference in viral abundances between mitomycin C-treated and untreated incubation samples during this period was assumed to be the result of lysogenic phage production. After this initial period, viral abundances remained comparatively stable in the mitomycin C-treated samples but increased in the controls of some experiments as a result of increasing bacterial biomass and lytic phage production.

In the January 1995 experiments, mitomycin C was also added to untreated incubation samples after the beginning of the experiment, when bacteria showed vigorous growth (Fig. 3). The phage abundances in these incubations reached levels

FIG. 1. Effect on bacterial and viral abundances of filtration of natural seawater samples through $1.0\text{-}\mu\text{m}$ -pore-size filters and concentration of the remaining particulate matter by using a $0.2\text{-}\mu\text{m}$ -pore-size filter. Bacterial abundances in the $>0.2\text{-}\mu\text{m}$ size fraction are expressed following dilution of the concentrate to its original volume. NSW, unfiltered, natural seawater; $<1.0\text{-}\mu\text{m}$, seawater filtered through a $1.0\text{-}\mu\text{m}$ -pore-size filter; $<0.2\text{-}\mu\text{m}$, $<1.0\text{-}\mu\text{m}$ size fraction filtered through $0.2\text{-}\mu\text{m}$ -pore-size filter; $>0.2\text{-}\mu\text{m}$, abundance of bacteria in the $<1.0\text{-}\mu\text{m}$ size fraction following concentration with a $0.2\text{-}\mu\text{m}$ -pore-size filter and dilution to the original volume with ultrafiltrate (11 Nov 94), microwaved (26 Nov 94), or autoclaved (all other dates) seawater. Stations: A, pier; B, 25 km offshore; C, 50 km offshore; D, 100 km offshore. x, no samples collected. When error bars are not shown, the standard deviation calculated from duplicate incubations was too small to be illustrated.

FIG. 2. Viral and bacterial abundances expressed relative to those at the beginning of the experiments. Seawater was collected 50 km offshore on 19 December 1994. UV-C doses were administered in a single pulse. The final mitomycin C concentration was $1.0 \mu\text{g ml}^{-1}$. When error bars are not shown, the standard deviation calculated from duplicate incubations was less than the width of the symbols.

higher than those in incubations of samples which received mitomycin C at the start of the experiments. About 12 h after mitomycin C was added to growing bacteria, the viral abundances were higher than those in the control incubations. Later, viral abundances in the controls increased and were similar to those in the mitomycin C-treated samples.

Viral dynamics and UV-C treatment. During the first 24 h in the experiments when UV-C was used, at least one dose resulted in viral numbers that were significantly higher than those in the control incubations (Fig. 2). However, the UV-C dose that yielded the highest viral abundances was not the same in all experiments. In the experiment started on 26 November 1994, the highest viral abundance occurred following a dose of $100,000 \mu\text{J cm}^{-2}$, whereas in the experiment from 19 December 1994 a dose of $30,000 \mu\text{J cm}^{-2}$ produced the highest viral abundances. Although viral abundances were sometimes higher with the UV-C treatments than in the controls, viral production was always lower with the UV-C treatment than with the mitomycin C treatment (Table 2).

Burst size. At each sample date, the burst size was estimated separately for all visibly infected bacteria (minimum burst size) and for cells which were completely filled with viral particles (maximum burst size). The minimum burst size in the mitomycin C incubations ranged from 11 to 29 (average, 21 ± 6.0), and the maximum burst size ranged from 21 to 45 (average,

30 ± 7.8) (Table 2). The minimum burst size of bacteria in the seawater collected for the experiments ranged from 15 to 34 (average, 24 ± 7.3), and the maximum burst size ranged from 23 to 54 (average, 35 ± 10.3) (Table 1). The burst sizes in the untreated seawater were significantly, but only slightly, higher than those with the mitomycin C treatments (paired t test, two tailed; P of 0.0001).

Percentage of lysogenic bacteria. After 10 to 24 h of incubation, the viral abundances were higher with the mitomycin C treatment than in the control incubations, and the difference was attributed to phage production as a result of induction of lysogenic bacteria. The number of induced bacteria was calculated as $(V_{mc} - V_c)/BS$, where V_{mc} is viral abundance in samples with the mitomycin C treatments, V_c is viral abundance in control incubations, and BS is the burst size. Burst sizes were determined in the mitomycin C-treated incubations at the time when the maximum differences occurred between viral abundances in the mitomycin C-treated and control incubations. We estimated that between 0.07 and 4.4% of the bacteria present at the start of the experiments (mitomycin C-treated incubations) were lysogenic (average, $1.5 \pm 1.4\%$) (Table 2). The percentage of lysogenic cells did not differ significantly (t test, two tailed; $P > 0.3$) between samples collected at the

FIG. 3. Viral and bacterial abundances expressed relative to their abundances at the beginning of the experiments. Seawater was collected 25 km offshore on 17 January 1995. The final mitomycin C concentration was $1.0 \mu\text{g ml}^{-1}$. When error bars are not shown, the standard deviation calculated from duplicate incubations was less than the width of the symbols. In one treatment (triangles), mitomycin C was added to control cultures after 11 h of incubation, after the bacteria had entered the exponential growth phase.

TABLE 2. Estimation of percentages of lysogenic bacteria in the total bacterial community based on induction by mitomycin C

Date of sampling	Station ^a	$V_{mc} - V_c^b$	$V_{uv} - V_c^{b,c}$	Burst size ^{d,e}		Bacterial abundance (10^6 ml^{-1}) ^{e,f}	% lysogenic bacteria ^g	
				Minimum	Maximum		Maximum burst size	Minimum burst size
11 Nov 94	A	3,275,000	250,000	21 ± 2.8	31 ± 5.0	3.51 ± 0.252	3.0	4.4
26 Nov 94	A	1,850,000	1,320,000	17 ± 4.2	25 ± 2.8	3.50 ± 0.201	2.1	3.1
06 Dec 94	A	4,100	ND	23 ± 2.8	27 ± 2.1	0.21 ± 0.010	0.07	0.08
19 Dec 94	C	8,200	6,600	29 ± 5.0	37 ± 5.7	0.10 ± 0.009	0.22	0.28
03 Jan 95	A	9,100	3,900	19 ± 2.1	29 ± 2.8	0.10 ± 0.021	0.31	0.48
17 Jan 95	C	53,000	ND	11 ± 0.0	22 ± 2.8	0.11 ± 0.010	2.2	4.4
17 Jan 95	B	26,000	ND	15 ± 7.1	21 ± 6.4	0.16 ± 0.007	0.77	1.1
26 Jun 95	D	61,200	ND	25 ± 8.2	36 ± 5.9	0.22 ± 0.096	0.77	1.1
28 Jun 95	C	65,580	ND	28 ± 5.6	45 ± 11.2	0.18 ± 0.035	0.80	1.6

^a Stations: A, pier; B, 25 km offshore; C, 50 km offshore; D, 100 km offshore.

^b V_{mc} , viral abundance (milliliter⁻¹) in the mitomycin C-treated incubation; V_c , viral abundance (milliliter⁻¹) in the control incubation.

^c V_{uv} , maximum viral abundance (milliliter⁻¹) in the UV-C-treated incubation. The UV-C doses ($\mu\text{J cm}^{-2}$) that produced the greatest viral abundance (date of sampling is indicated in parentheses) were 40,000 (11 November 94); 100,000 (26 November 94); 30,000 (19 December 94); and 50,000 (03 January 95). ND, not determined.

^d Burst size was estimated from the average number of viral particles seen in all visibly infected cells (minimum) or from the average number of viruses in cells that were completely filled with viral particles (maximum).

^e Values are expressed as the means ± standard deviations calculated for duplicate incubations.

^f Bacterial abundance at the onset of the experiments (mitomycin C treatment).

^g Values are calculated as $\{(V_{mc} - V_c)/BS/BA\} \times 100$, where BS is burst size and BA is bacterial abundance.

pier (0.07 to 4.4%) and at the more offshore stations (0.22 to 4.4%).

DISCUSSION

In these experiments it was only possible to induce viral production in a small proportion of the natural bacterial community by the addition of mitomycin C or by exposure to UV-C. There are two possible explanations for these results. The first is that the treatments were unable to induce the majority of cells that were lysogenized, in which case the proportion of the community that was lysogenized would have been underestimated. Although the possibility that the treatments were inadequate to induce most of the lysogenized cells cannot be ruled out, there is no evidence that this was the case. Given the current state of knowledge regarding triggering induction in lysogenized bacteria, it is reasonable to assume that most lysogenized bacteria were induced. At the present time there is no basis to reject the alternative explanation that the majority of bacteria in our incubations were not lysogenized. At the very least we can state that the majority of growing bacteria in our incubations could not be induced by treatment with mitomycin C or UV-C radiation.

The data presented in this paper show that in nine experiments performed over a 7-month period, lysogenic bacteria induced by mitomycin C ranged from 0.07 to 4.4% of the total bacterial community (Table 2). A low percentage of lysogens is also supported by the observation that bacterial abundances with the mitomycin C treatments did not decrease significantly. A strong decrease in bacterial abundance would be expected if the induction of lysogens had caused significant bacterial mortality. Moreover, the proportion of cells that were induced did not differ between nearshore and the more oligotrophic offshore stations. In contrast, the percentage of lysogenic cells found in bacterial isolates was higher in oligotrophic than in eutrophic environments (10) and was much higher (on average, 43% were lysogenic or bacteriocinogenic) than we observed for the entire bacterial community. There have been reports of high viral production induced by mitomycin C in natural bacterial communities (11, 29); however, because details from these investigations have yet to be published, direct comparison with the results from our studies is not possible.

Induction due to UV light. UV-C induced phage production in some experiments, but the addition of mitomycin C always resulted in greater viral production. Mitomycin C was also found to be a more powerful inducing agent than UV-C in lysogenic strains of *Listeria* spp. (13). Moreover, the UV-C dose yielding maximum viral production varied between experiments (Table 2). The reason for the variation in the UV-C dose required to maximize the induction of the lytic cycle is unknown but may have resulted from differences in the nutritional states among the microbial communities, which can affect the dosage of UV-C required to induce lysogenic bacteria (15).

It is well-known that damage to DNA by UV-C can induce lysogenic bacteria, however, as UV-C does not reach the Earth's surface this is of little ecological relevance. Yet DNA can also be damaged by UV-B and UV-A (20), which reaches the surface of the Earth and penetrates to considerable depth in seawater (23). For example, UV-B and solar radiation can induce DNA damage in marine phytoplankton and bacteria (9, 12). Furthermore, RecA activity, which cleaves a repressor and induces prophages to enter the lytic cycle, is known to occur in marine bacterioplankton in response to UV-B stress and DNA damage (16). Consequently, it is reasonable to assume that light-mediated induction as observed in the present study can also occur in situ. Nonetheless, our results suggest that sunlight will be a minor contributor to overall phage production, given the small proportion of the bacterial community that was induced by UV radiation.

Induction due to sample handling and vigorous bacterial growth. Bratbak et al. (4) reported that viral abundances can increase dramatically within minutes to hours after sample collection and argued that lysogenic phage production might be responsible for this observation. Viral numbers did not increase following filtration through 1.0- or 0.2- μm -pore-size polycarbonate filters (Fig. 1), a process which was completed in about 40 to 90 min. This result indicates that lysogenic phage production did not contribute significantly to viral abundance during preparation of the incubations.

With phage lambda, lysogens can be induced by rapid bacterial growth (7). In some experiments viral abundances increased in the control samples after bacteria entered the exponential growth phase. However, even when mitomycin C was

added to exponentially growing cells, there was a stimulation of viral production, indicating that lysogens remained in the bacterial community (Fig. 3). The number of viruses that were released indicated that about the same proportion of cells were lysogenized as were present prior to the bacteria entering the exponential growth phase. If rapid growth had induced some of the lysogens, then fewer lysogens would remain, and we would have expected a smaller proportion of the community to be induced by the addition of mitomycin. These data provide no evidence that higher growth rates induced lysogenized cells.

The fact that increases in viral abundance occurred only when bacteria exceeded ca. 10^6 cells ml^{-1} (Fig. 3) is consistent with increased viral production via lytic infection when bacterial abundance, and hence encounter rates, increased between phages and bacteria. This would result in more frequent infection events by lytic viruses and rapid viral propagation. Similar observations have been made for other lytic viral systems (34). These results also emphasize the importance of reducing viral and bacterial abundances before attempting to induce lysogenic phage production. Otherwise, any production of viruses via induction of lysogens may be swamped by viruses produced by lytic infection. This is particularly true because mitomycin C may also affect rates of lytic viral production, making lytic infection difficult to control for other than by reducing encounter rates.

Lysogenic phage production and bacterial mortality. The proportion of the bacterial community that was estimated to be lysogenic ranged from 0.08 to 4.4%, when the average number of viruses determined from all visibly infected cells was used to estimate burst sizes (Table 2). However, estimating burst size by averaging the number of viral particles in all visibly infected cells (minimum burst size) will overestimate the proportion of cells that are lysogenic, because, in many cases, the number of viruses per cell will be higher before lysis takes place. Therefore, it is more realistic to estimate burst sizes by averaging the number of viruses in cells which were filled with viral particles (maximum burst size). This method results in estimates of 0.07 to 3.0% of the bacterial community being lysogenic. Regardless of which estimate of burst size is used, it is clear that a relatively small proportion of the bacterial community could be induced by mitomycin C.

A steady-state model was used to estimate the bacterial mortality that potentially results from induction of lysogenic cells. The model assumes that a bacterial cell divides, one daughter cell dies, and the other survives and divides again. Therefore, something which kills 50% of the bacteria is responsible for 100% of the mortality (21, 22). The induction of all lysogenic cells in the investigated samples (Table 2) would cause the lysis of 0.07 to 4.4% of the total bacterial community, representing 0.14 to 8.8% of the total mortality, assuming that the bacterial population doubles about once a day. It is more realistic that only a small proportion of the population would be induced at a given time; thus, induction of lysogenic cells does not seem to be a very important cause for bacterial mortality. Moreover, as estimates suggest that on average about 20% of the cells in natural bacterial communities are infected by viruses (27), lysogenic cells would represent a small percentage of the total infected cells.

The maximum number of viruses that could be produced as the result of induction of lysogens was estimated from bacterial abundances, the maximum determined burst size, and the higher percentages of lysogenic cells for each sample date (Table 2). If all of the lysogens were induced, the range in the number of viruses that would be produced would be 6.8×10^4 to 5.1×10^6 viruses ml^{-1} , or 0.44 to 73.9% (average, $17.2\% \pm 22.49\%$) of the standing stock of viruses, respectively. This

estimate does not represent the actual contribution of lysogenic to total phage production, since the percentages of bacteria that are induced at any given time and the lytic phage production are unknown. However, it suggests that lysogenic phage production would be an important contributor to total phage production only when the majority of the lysogenic cells are induced and lytic phage production is low. UV radiation and mitomycin C do not always induce the same phages (1). If we assume that UV-C and mitomycin C induced different phages, the proportion of lysogenized bacteria would still be small, ranging from 0.46 to 4.7% of the bacterial community, thus not significantly influencing the argument outlined above. These data are consistent with those of Wilcox and Fuhrman (35), who showed that lytic infection rather than induction of lysogenic bacteria was primarily responsible for the production of phages in the coastal waters of southern California.

This paper presents estimates of the percentage of cells that are lysogenic in natural marine bacterial communities. We found that induction of lysogenic bacteria is unlikely to be an important cause of mortality for marine bacteria in coastal waters of varying trophic status and is unlikely to contribute significantly to phage production. This interpretation is dependent on the assumption that mitomycin C can be used to induce most lysogenized bacteria in natural marine communities. At the present time this is a reasonable assumption given that in most studies lysogens appear to be induced by mutagens such as mitomycin C. Despite these caveats our results suggest that the effects of phages on mortality, nutrient flow, and species diversity in microbial food webs depend largely on the lytic pathway of viral infection.

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