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Incidence of *Vibrio parahaemolyticus* Bacteriophages and Other *Vibrio* Bacteriophages in Marine Samples†

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Vibrio bacteriophages were isolated by enrichment from 177 of 643 samples of marine molluscan shellfish, crustaceans, seawater, and sediments. The predominant bacteriophage types isolated were specific for some strains of *Vibrio parahaemolyticus*. A high frequency of phage isolations was also observed with strains of agar-digesting vibrios (21 of 56) and psychrophilic vibrios (14 of 72) that were originally isolated from non-shellfish growing areas. No bacteriophages were isolated against *V. alginolyticus* and only rarely for *V. anguillarum* even though these were the two most abundant species found in near-shore environments. No *V. cholerae* phages were isolated. It was also determined from quantitative studies on the Pacific oyster (*Crassostrea gigas*) obtained from two environments in Washington and Oregon that the titers of *V. parahaemolyticus* bacteriophages increased with increasing seasonal water temperatures and that this was proportional to the increase in numbers of mesophilic vibrios and not with the incidence of *V. parahaemolyticus*. Titers of *V. parahaemolyticus* bacteriophages occasionally exceeded 10^6 per g of oyster during the summer months. Specific *V. parahaemolyticus* bacteriophages were also isolated from market seafoods and other marine samples that originated in cold environments where no mesophilic vibrios are expected to be found. The possibility that *V. parahaemolyticus* bacteriophages originate from *Vibrio* spp. other than *V. parahaemolyticus* and the role of these bacteriophages in the ecology of marine vibrios are discussed.

The ubiquity of bacteriophages in various environments has been demonstrated repeatedly. In the marine environment, bacteriophages have been isolated that lyse a variety of indigenous bacteria, including *Pseudomonas* spp. (17, 28, 29, 31), *Vibrio/Beneckea* spp. (5, 16, 17, 22, 26, 34; R. P. Saunders, J. R. Andolina, and P. K. Chen, Abstr. Annu. Meet. Am. Soc. Microbiol. 1972, V209, p. 220), *Cytophaga* spp. (8, 29), *Agrobacterium* spp. (2), *Photobacterium* spp. (13, 19, 27), and various nonmarine contaminants, particularly members of the *Enterobacteriaceae*. Enrichments using seawater, sediments, and various marine animal samples have been successfully used as a source of bacteriophages. A wide variety of marine bacteriophages have been described and include different morphological types (14, 15, 17, 22, 35). Moreover, many of these bacteriophages are obligately marine in that they are psychrophilic and have a requirement for salts at marine concentrations for infection and growth (19, 31, 35).

Many *Vibrio* spp., including human, fish, and invertebrate pathogens, occur commonly and abundantly in near-shore marine environments, and thus it is not surprising that vibrio bacteriophages have frequently been isolated. Spencer (29) isolated several bacteriophages, some of which lysed marine vibrios, and Smith and Krueger (26) reported on the isolation of a vibrio phage from San Francisco Bay sediment. Also, Hidaka (14) isolated three vibrio DNA phages from seawater off the coast of Japan, and Kakimoto and Nagatomi (17) isolated two heat-resistant vibrio phages from Kinko Bay water. Similarly, a bacteriophage active against *V. marinopraescens* was isolated from Indian Ocean sediments (16). *V. natriegens* (*Beneckea*) bacteriophages were found to be widely distributed in near-shore environments, particularly in salt marshes (34).

The first isolation of *V. parahaemolyticus* phages was reported by Nakanishi et al. in 1966 (22). The three bacteriophages described were isolated from seawater, human feces, and a lysogenic strain of *V. parahaemolyticus* and were found to be distinctly different based on plaque morphology, host range, and serological specificity. Other *V. parahaemolyticus* phages have

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been isolated by Baross and Liston (5), Saunders et al. (Abstr. Annu. Meet. Am. Soc. Microbiol. 1972, V209, p. 220), and Sklarow et al. (25).

All of the reports of vibrio bacteriophage isolations were based on qualitative enrichments, and thus there is no indication as to the numbers of specific bacteriophage present in marine environments. Moreover, it is not known whether or not, and to what extent, bacteriophage populations are influenced by various chemical and physical factors and by the resident bacterial population. In this report the numbers of *V. parahaemolyticus* and other vibrio bacteriophages were measured from various inshore marine samples and related to the seasonal incidence of different *Vibrio* spp.

MATERIALS AND METHODS

Source of bacterial strains. *V. parahaemolyticus* K and Sak strains were supplied by R. Sakazaki (National Institute of Health, Tokyo, Japan) and R. R. Colwell (Department of Microbiology, University of Maryland, College Park). *V. cholerae* strains and *V. parahaemolyticus* FC 1011 were supplied by R. R. Colwell. *V. parahaemolyticus* T 3980 and *V. alginolyticus* V-374 were supplied by H. ZenYoji (Tokyo Metropolitan Research Laboratory of Public Health, Shinjuku-ku, Tokyo, Japan). *V. anguillarum* strains 2.1 and V-2911 were obtained from E. J. Ordal (Department of Microbiology, University of Washington, Seattle). All other *Vibrio* strains including psychrotrophic strains, agar digesters, and luminous species were isolated throughout the course of this study from various samples of marine fauna, sediment, and seawater obtained from Washington and Oregon.

Isolation of marine vibrios. Samples of molluscan and crustacean shellfish, finfish, squid, seawater, and sediment were obtained from various marine environments and markets throughout Oregon and Washington. All samples were prepared as previously described (6), and samples of the Pacific oyster (*Crassostrea gigas*) and various clam and mussel species were blended with an equal weight to volume of 3.5% Rila marine salts (Rila Products, Teaneck, N.J.), whereas crustaceans and small oysters (*Ostrea lurida* and the Kumomoto variety of *C. gigas* frequently weigh under 2 g/animal) were blended at a weight-to-volume ratio of 1:5 with 3.5% Rila salts. Sample sizes of the various animals that were blended and used for the isolation of vibrios or vibrio bacteriophages depended on the species of animal and ranged from 20 to 400 g. Samples of small finfish (*Hypomesus pretiosus*) and squid (*Loligo opalescens*) were aseptically cut into 2- to 3-cm sections and blended at a 1:5 dilution with 3.5% Rila salts. Seawater and sediment samples were serially diluted in 3.5% Rila salts.

The vibrio populations enumerated in all samples were the total mesophilic counts (total vibrios at 37°C) and counts of *V. alginolyticus* and *V. parahaemolyticus*. In addition, a 15°C *Vibrio* count was made on Pacific oyster samples obtained from Purdy, Wash., and Yaquina Bay, Ore., using thiosulfate-citrate-bile-sucrose agar (TCBS, Difco). Total mesophilic counts and counts of *V. alginolyticus* and *V. parahaemolyticus* were determined with both a seawater starch agar (6) and thiosulfate-citrate-bile-sucrose agar, as outlined by Morris et al. (21). *V. parahaemolyticus* and *V. alginolyticus* were confirmed with the differentiating scheme as outlined by Morris et al. (21). The taxonomic scheme of Shewan et al. (24) for gram-negative bacilli was used to confirm all other presumptive vibrios.

The seasonal incidence of various vibrios was measured in Pacific oysters (*C. gigas*) obtained from Purdy, Wash., and Yaquina Bay, Ore.

Isolation of vibrio bacteriophage. Both a qualitative enrichment technique and a quantitative direct plating method were used to isolate bacteriophage. The enrichment procedure involved the addition of marine samples to log-phage cultures of host strains. Trypticase soy broth (BBL) supplemented with 2.5% NaCl or 3% Rila salts was used as the growth and enrichment medium. Marine samples were added at concentrations between 1 and 10% in 250 ml of broth, except seawater samples, which were added in equal volume to 100 ml of double-strength enrichment broth. The enrichment mixtures were incubated at 22°C with slow aeration for 24 to 72 h and then allowed to settle for 48 h. Similarly, this technique was applied to estimate the most probable number of bacteriophages, using a three-tube dilution series. This most-probable-number technique was used when the suspected levels of bacteriophage were low. After settling, all samples were centrifuged for 15 min at 12,500 × *g* (Sorvall RC-2B refrigerated centrifuge). The supernatants from all samples were tested for the presence of bacteriophage by spotting 0.01 ml from each dilution onto the surface of an agar plate overlaid with the host strain.

The direct bacteriophage isolation procedure was used on shellfish only and consisted of making dilutions of homogenates (to 10^{-6}) in buffer (Na_2HPO_4, 9.5 g; KH_2PO_4, 3.0 g; NaCl, 25 g; 0.1 M $MgSO_4$, 10 ml; 0.1 M $CaCl_2$, 10 ml; distilled water, 1 liter; adjusted to pH 7.5), and the contents of each dilution tube were centrifuged at 12,500 × *g* for 15 min. For enumeration of virulent bacteriophage, a 1-ml sample from each dilution, in duplicate, was plated with the host strain, using the soft-agar overlay technique of Adams (1). The soft-agar overlay was prepared with phage buffer supplemented with 0.7% agar. The plating medium used for the enumeration of bacteriophage was either nutrient agar (Difco) supplemented with 0.5% potato starch (Baker) and 2.5% NaCl or Lib-X agar (4) supplemented with 0.5% starch. All plates were incubated for 24 to 48 h at 22°C.

RESULTS

The occurrence of marine vibrio bacteriophages was determined by inoculation of log-phase cultures of different species and strains of vibrios with various marine environmental samples. Table 1 summarizes these data and shows a high incidence of bacteriophage isolations from marine animals and a much lower incidence from seawater and sediments. The percentage of

TABLE 1. *Incidence of isolation of vibrio bacteriophage from enrichments with marine environmental samples*

Strain tested	No. of enrichments											
	Oysters[a]		Clams[a]		Crab		Shrimp		Seawater		Sediments	
	NT[b]	NP[b]	NT	NP	NT	NP	NT	NP	NT	NP	NT	NP
V. parahaemolyticus												
Japanese strains												
ATCC 17802	28	0	8	0	2	0	0	0	5	0	5	0
K-3	15	13	6	4	2	2	1	0	4	0	4	1
K-4	62	57	8	6	9	3	3	0	12	0	7	1
K-12	14	0	2	0	0	0	0	0	0	0	0	0
SAK-4	16	0	5	0	1						3	0
SAK-5	5	0	3	0	1	0	0	0	0	0	3	0
SAK-6	8	6	5	3	1	1	0	0	4	0	4	0
SAK-9	23	19	7	4	2	1	1	1	4	0	5	1
SAK-11	6	4	2	2	0	0	0	0	0	0	0	0
SAK-18	10	0	5	0	0	0	0	0	0	0	0	0
SAK-34	4	1	0	0	0	0	0	0	0	0	0	0
T3980-1	10	7	2	1	0	0	0	0	1	0	1	0
U.S. strains												
81BOH4	5	0	2	0	0	0	0	0	4	0	6	0
FC1011	8	0	4	0	1	0	0	0	3	0	3	0
V. alginolyticus												
ATCC 17749	28	0	8	0	0	0	0	0	6	0	6	0
V-374	10	0	4	0	0	0	0	0	0	0	0	0
V. anguillarum												
2.1	15	0	4	1	1	1	0	0	5	1	2	0
V-2911	5	0	0	0	1	0	0	0	5	0	2	0
SOY-2A	6	0	0	0	0	0	0	0	0	0	0	0
V. cholerae (3 strains)	12	0	6	0	0	0	0	0	0	0	0	0
Psychrophilic vibrios (6 strains)	53	13	4	0	3	0	0	0	8	1	4	0
Agar-digesting vibrios (4 strains)	47	19	0	0	0	0	0	0	0	0	9	2
Luminous vibrios (2 strains)	6	1	3	0	0	0	0	0	0	0	5	0

[a] Oyster species were *C. gigas* (including the Kumamoto variety) and *O. lurida*; clam species were *V. japonica* and *M. arenarea*. Oysters and clams were obtained from various environments in Washington and Oregon.

[b] NT, Total number of enrichments; NP, total number of enrichments yielding bacteriophages.

positive phage isolations was 35% from oysters (n = 396), 24% from clams (n = 88), 33% from crabs (n = 24), 20% from shrimps (n = 5), 3% from seawater (n = 61), and 7% from sediments (n = 69). These data, however, are somewhat misleading, since the frequency of isolation of bacteriophage for some strains of *V. parahaemolyticus* and various environmental strains of psychrophilic and agar-digesting vibrios was very high. Conversely, there was either a very low incidence or no positive phage isolations with some of the most commonly occurring species such as *V. alginolyticus* and *V. anguillarum*. Specifically, 37% of a total of 375 enrichments with *V. parahaemolyticus* were positive for bacteriophage. No phages were isolated from 62 enrichments with *V. alginolyticus* and 18 enrichments with *V. cholerae*, and only 3 of 46 enrichments yielded *V. anguillarum* bacteriophages. No virulent bacteriophages were isolated for some strains of *V. parahaemolyticus* such as ATCC 17802 and Sak-4, whereas 88% of all oyster and 70% of all clam enrichments with strains K-3, K-4, Sak-6, Sak-9, Sak-11, and T3980-1 yielded virulent bacteriophages. Vibrio phages were rarely isolated from seawater and sediments, even though quite high vibrio populations were frequently detected.

The correlation between the presence of mesophilic *Vibrio* spp., including *V. alginolyticus* and *V. parahaemolyticus*, with specific *V. parahaemolyticus* bacteriophages was not always unequivocal. It was not unusual, for example, to isolate *V. parahaemolyticus* phages from market seafood samples that had no detectable levels of mesophilic vibrios (Table 2). This was particularly true in the case of the snow crab (*Chionoecetes bairdii*) and king crab (*Parali-*

thodes camtschatica) samples, which originated from the cold waters of Alaska. It is not suspected that *V. parahaemolyticus* and other mesophilic vibrios could survive during any time of the year, since these species are known to be quite sensitive to refrigerator temperatures (10). It is interesting that *V. parahaemolyticus* bacteriophages were invariably isolated from market shucked oysters and frozen clams even though mesophilic vibrios and *V. parahaemolyticus* were infrequently isolated from these samples.

The apparent ubiquity of vibrio bacteriophages in various bivalve mollusks prompted further investigations into the relationship between the actual numbers of phage and the numbers of specific species of mesophilic vibrios. The host vibrios used were *V. parahaemolyticus* K-4, and $TC_{11}E_2A$, an agar-digesting vibrio isolated from Puget Sound sediments. These results are summarized in Table 3 and definitely show that K-4 bacteriophages occur in very high numbers from oysters and clams obtained from several shellfish growing areas in Oregon and Washington and in market samples. It is apparent from these data that, in general, a high incidence of K-4 bacteriophage coincided with high numbers of mesophilic vibrios and not with *V. parahaemolyticus* counts, which were frequently very low or undetectable. The highest incidence of K-4 bacteriophage, however, occurred in a clam sample (*Venerupsis japonica*) that was inculpated as the cause of an outbreak of gastroenteritis in Washington State. This

TABLE 2. *Comparison of the numbers of mesophilic vibrios, V. alginolyticus, and V. parahaemolyticus with the presence of specific V. parahaemolyticus K-4 bacteriophages from enrichments with market seafood samples*

Product	No.	Total vibrios/g			Presence of bacteriophage
		Mesophilic vibrios	*V. alginolyticus*	*V. parahaemolyticus*	
Shucked oysters (*C. gigas*)	1	91,000	100	<10	+
	2	2,000	100	<10	+
	3	100	<10	<10	+
	4	490	<10	<10	+
	5	1,000	30	<10	+
	6	750	<10	<10	+
	7	800	<10	<10	+
	8	230,000	1000	20	+
	9	NT[a]	80	10	+
	10	NT	50	<10	+
	11	NT	40	<10	+
	12	NT	15	<10	+
Shucked oysters (*O. lurida*)	1	200	50	<10	+
	2	50,000	20,000	340	+
	3	35,000	3,000	10	+
Clams (live) (*V. japonica*)	1	2,500	250	<10	−
	2	NT	10	<10	+
	3	NT	300	<10	+
Clams (frozen) (*V. japonica*)	1	NT	<10	<10	+
Crab meat (fresh) (*Cancer magister*)	1	4,000	<10	<10	−
	2	400	<10	<10	−
	3	700	200	<10	−
	4	<10	<10	<10	+
	5	NT	<10	<10	−
Snow crab meat (*C. bairdii*)	1	NT	<10	<10	+
	2	NT	<10	<10	+
King crab meat (*P. camtschatica*)	1	<10	<10	<10	+
	2	<10	<10	<10	+
Squid (whole, fresh) (*L. opalescens*)	1	10	<10	<10	+
	2	250	<10	<10	+
	3	NT	<10	<10	−
Smelt (whole, fresh) (*H. pretiosus*)	1	60	<10	<10	−
	2	NT	<10	<10	−

[a] NT, Not tested; in these samples the vibrio numbers were determined using an incubation temperature of 43°C, which enumerates only *V. alginolyticus* and *V. parahaemolyticus*.

TABLE 3. *Comparison of total mesophilic vibrios, V. alginolyticus, and V. parahaemolyticus counts with the numbers of specific vibrio bacteriophages isolated from marine animals*

Sample	Source	Total vibrios/g of sample			Specific host strain and no. of bacteriophage/g of sample	
		Mesophilic vibrios	*V. alginolyticus*	*V. parahaemolyticus*	K-4[a]	$TC_{11}E_2A$[a]
Pacific oysters (*C. gigas*)	Purdy, Wash.	800	10	<10	400	<10
	Purdy, Wash.	5,000	100	<10	1,650	NT[b]
	Purdy, Wash.	6,400	400	<10	55,000	5,000
	Purdy, Wash.	17,000	10,000	1,100	69,000	2,200
	Purdy, Wash.	10,000	8,000	1,000	400,000	2,800
	Purdy, Wash.	4,000	1,100	<10	100,000	NT
	Purdy, Wash.	5,000	100	<10	1,650	5,000
	Market (shucked)	22,000	600	50	11,000	NT
	Market (shucked)	6,500	15	<10	15	<10
	Market (shucked)	12,000	135	<10	400	NT
	Market (shucked)	60,000	1,500	300	80,000	1,800
	Big Beef, Wash.	20	5	<10	58	30
	Big Beef, Wash.	100,000	10	<10	18,000	<10
	Shelton, Wash.	10	<10	<10	4,300	590
	Shelton, Wash.	9,000	1,000	200	<10	<10
	Seabeck, Wash.	45,000	200	<10	5,000	5,000
	Astoria, Ore.	250	200	<10	<10	<10
	Yaquina Bay, Ore.	14,000	8,000	1,200	2,000	NT
	Yaquina Bay, Ore.	120,000	4,000	300	55,000	NT
	Tillamook Bay, Ore.	700	<10	<10	<10	NT
Olympia oysters (*O. lurida*)	Shelton, Wash.	15,000	100	<10	400	NT
	Shelton, Wash.	200	50	<10	50	10
	Yaquina Bay, Ore.	450,000	300,000	50,000	>50,000	NT
	Yaquina Bay, Ore.	NT	44,000	8,000	80,000	NT
Clams (*V. japonica*)	Purdy, Wash.	7,000	200	10	50	NT
	Purdy, Wash.	30	25	<10	20	<50
	Purdy, Wash.	60	<10	<10	<10	10
	Seabeck, Wash.	1,090,000	100,000	16,000	500,000	8,000
Clams (*M. arenarea*)	Yaquina Bay, Ore.	1,500	50	<10	400	NT
	Yaquina Bay, Ore.	18,000	6,000	50	2,000	NT
	Yaquina Bay, Ore.	200	<10	<10	<10	NT
Mussels (*Mytilus edulis*)	Yaquina Head, Ore.	80	<10	<10	1,200	NT
Crab (picked meat)	Market sample	700	200	10	9,600	NT
(*C. magister*)	Market sample	<10	<10	<10	150	NT

[a] K-4 is *V. parahaemolyticus*; $TC_{11}E_2A$ is a psychrotrophic agar-digesting vibrio isolated from a sediment sample obtained from Puget Sound, Wash.

[b] NT, Not tested.

sample of clams also contained significantly high levels of *V. parahaemolyticus*. The numbers of $TC_{11}E_2A$ phages in these shellfish samples were considerably less than the incidence of K-4 phages; however, it is apparent that more than one kind of bacteriophage exist simultaneously in these samples.

The relationship between the actual incidence of *V. parahaemolyticus* bacteriophages and the numbers of specific species of vibrios was investigated by using Pacific oysters (*C. gigas*) obtained from various shellfish growing areas on a regular seasonal basis. The incidence of the total mesophilic vibrio population, *V. parahaemolyticus*, *V. alginolyticus*, and the total vibrios as measured at 15°C were also quantitatively measured in the same samples. These data are shown in Fig. 1. Clearly, oysters harbor a high vibrio population throughout the year, which is composed of predominately psychrotrophic vibrios during late fall to early spring (<5 to 10°C), and a mixture of psychrotrophic and mesophilic vibrios, including *V. alginolyticus* and *V. parahaemolyticus*, during the summer months (>10 to 30°C). *V. anguillarum* comprises a significant proportion of the psychrotrophic and mesophilic vibrios; however, the greatest portion of these vibrios has not been delineated into definable

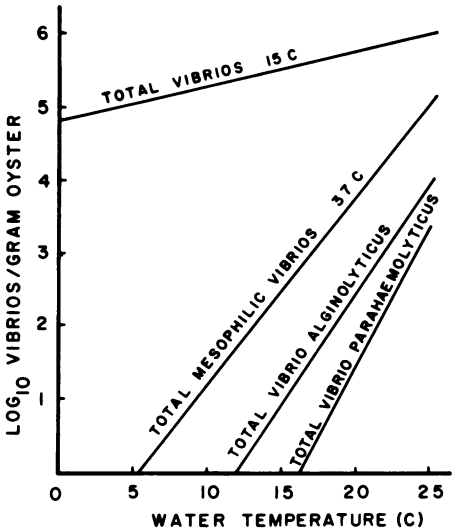

FIG. 1. *Relationship between the seasonal water temperature and the total numbers of psychrotrophic vibrios, isolated at 15°C (n = 15; r = 0.80); total mesophilic vibrios, isolated at 37°C (n = 81; r = 0.86); and V. alginolyticus (n = 88; r = 0.82) and V. parahaemolyticus (n = 28; r = 0.74), isolated from Pacific oysters (C. gigas) obtained from Purdy and Big Beef, Wash., and Yaquina Bay, Ore. (These linear patterns of incidence were determined by regression analysis.)*

species. The incidence of K-4 bacteriophage also shows a seasonal pattern similar to the seasonal cycle seen for mesophilic vibrios. *V. parahaemolyticus* K-4 bacteriophages found in oysters increase proportionally with increasing water temperature (Fig. 2) and thus are related to the incidence of the mesophilic vibrio population and not to the psychrotrophic vibrios. Indeed, of the different species of vibrios, the incidence of K-4 phage shows a high statistical correlation ($r = 0.92$) to the mesophilic vibrios but not to the *V. parahaemolyticus* population (Fig. 3). Frequently the levels of K-4 bacteriophage exceeded 10^3/g of oyster even though there was no detectable population of either *V. alginolyticus* or *V. parahaemolyticus* in these samples.

DISCUSSION

The frequent isolations and the high numbers of bacteriophages associated with inshore marine animals suggest that these viruses play an important role in the ecology of marine vibrios. The seasonal incidence of bacteriophages in molluscan shellfish capable of lysing specific strains of *V. parahaemolyticus* was shown to be proportionately related to the incidence of mesophilic vibrios, which in turn was influenced by the ambient water temperatures. High levels of *V. parahaemolyticus* bacteriophages were detected in samples of shellfish that did not harbor detectable levels of mesophilic vibrios. Moreover, *V. parahaemolyticus* phages were frequently isolated from crustaceans such as the King crab and the Snow crab that reside in waters that are permanently cold (<5°C), thus strongly suggesting that the origin of these *V. parahaemolyticus* phages is not indigenous ly-

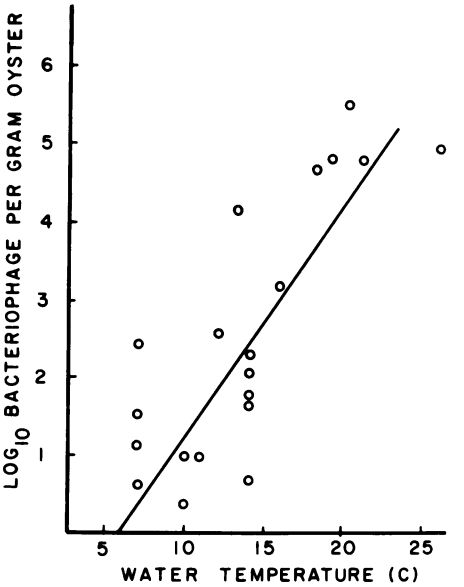

FIG. 2. *Correlation between the seasonal water temperature and the total numbers of specific V. parahaemolyticus K-4 bacteriophages isolated from the Pacific oyster, C. gigas (r = 0.80).*

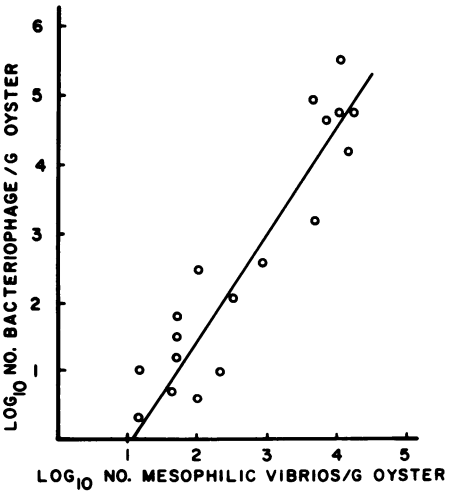

FIG. 3. *Correlation between the total numbers of mesophilic vibrios and the total numbers of specific V. parahaemolyticus K-4 bacteriophages isolated from the Pacific oyster, C. gigas (r = 0.92).*

sogenic strains of *V. parahaemolyticus*. This is contrary to the popular assumption that the chief requisite for bacteriophage isolation is the presence of the specific host organism. Indeed, this is generally true, and *Escherichia coli* and other *Enterobacteriaceae* phages are readily isolated from sewage (3, 18) and from sewage-polluted environments (18, 20). Similarly, specific bacteriophages have been isolated against indigenous spoilage bacteria from various nonmarine foods (32, 33) and from finfish (12). The frequent isolation of bacteriophages in marine sediments against many different bacterial genera reflects the complex and extensive nature of the microbial flora in these samples.

Obviously, the origin of these bacteriophages is obscurred because of our inadequate knowledge concerning the types, numbers, and activity of marine vibrios and their association with marine animals. Reliable taxonomic and physiological information exists only for the human and fish pathogenic species such as *V. parahaemolyticus*, *V. alginolyticus*, and *V. anguillarum* (9, 11, 30). Among these species, only *V. alginolyticus* is significantly related to *V. parahaemolyticus* to the extent that they could share common phages. However, no *V. alginolyticus* bacteriophages were ever isolated from marine samples, and none of the strains of this species tested was found to harbor prophages that could lyse *V. parahaemolyticus*. Also, like *V. parahaemolyticus*, *V. alginolyticus* is present only during the summer months, and invariably high populations of *V. parahaemolyticus* phages precede the rise in incidence of *V. alginolyticus* that occurs during the summer. Again, the obvious explanation is that *V. parahaemolyticus* phages originate from *Vibrio* spp. other than *V. parahaemolyticus* or *V. alginolyticus*. Supportive evidence showing that some of these bacteriophages were psychrophilic and incapable of replication at temperatures above 25 to 30°C was presented earlier (7).

The species of vibrios within the psychrotrophic and mesophilic population found in oysters (Fig. 1) are not adequately delineated. There are a number of psychrotrophic and agar-digesting vibrios, for example, that have not been identified. Moreover, within the *V. anguillarum* population, wide genotypic and phenotypic variations have been encountered (23), suggesting that specification within this group of marine vibrios might be impossible and that a continuum of genetically related organisms may exist. The high correlation of *V. parahaemolyticus* bacteriophages with the incidence of mesophilic vibrios indicates that one or more of these undelineated species of vibrios can serve as hosts for the phages. If this is correct, then some interesting problems can arise, particularly regarding the possibility that vibrios that might be marginally related to *V. parahaemolyticus* can share common bacteriophages and perhaps exchange genetic information.

The high incidence of bacteriophages in Pacific oysters indicates that phage replication may be occurring within these animals. This is consistent with the fact that vibrio bacteriophages were rarely isolated from the overlying water. It is presumed that these phages can lyse and genetically alter populations of related bacteria such as *Vibrio* spp., particularly in environments where these host organisms are present in high numbers and are growing. The Pacific oyster, and the guts of other invertebrates and vertabrates, may be suited to environments which favor bacteria-virus and bacteria-bacteria interactions. The actual roles of these bacteriophages in the ecology of bacteria and whether or not transduction is taking place in situ await further study.

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