Effect of Temperature on Adhesion of *Vibrio* Strain AK-1 to *Oculina patagonica* and on Coral Bleaching

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Laboratory aquarium experiments demonstrated that *Vibrio* strain AK-1 caused rapid and extensive bleaching of the coral *Oculina patagonica* at 29°C, slower and less-complete bleaching at 23°C, and no bleaching at 16°C. At 29°C, the application of approximately 100 *Vibrio* strain AK-1 cells directly onto the coral caused 50 and 83% bleaching after 10 and 20 days, respectively. At 16°C, there was no bleaching, even with an initial inoculum of 1.2×10^8 bacteria. To begin to understand the effect of seawater temperature on bleaching of *O. patagonica* by *Vibrio* strain AK-1, adhesion of the bacteria to the coral as a function of temperature was studied. Inoculation of 10^7 *Vibrio* strain AK-1 organisms into flasks containing 20 ml of seawater at 25°C and a fragment of *O. patagonica* resulted in net levels of bacterial adhesion to the coral of 45, 78, and 84% after 2, 6, and 8 h, respectively. The adhesion was inhibited 65% by 0.001% D-galactose and 94% by 0.001% methyl-β-D-galactopyranoside (β-M-Gal). After the incubation of *Vibrio* strain AK-1 with the coral for 6 h, 42% of the input bacteria were released from the coral with 0.01% β-M-Gal, compared to less than 0.2% when β-M-Gal was present during the adhesion step. Adhesion did not occur when *Vibrio* strain AK-1 was grown at 16°C, regardless of whether the corals were maintained at 16 or 25°C, whereas bacteria grown at 25°C adhered to corals maintained at 16 or 25°C. Bacteria grown at 25°C adhered avidly to Sepharose beads containing covalently bound β-D-galactopyranoside but failed to bind if grown at 16°C. These data suggest that elevated seawater temperatures may cause coral bleaching by allowing for the expression of adhesin genes of *Vibrio* strain AK-1.

Coral bleaching is the disruption of symbioses between coral hosts and photosynthetic microalgal endosymbionts (7), referred to as zooxanthellae. The loss of pigmented zooxanthellae causes a coral to lose color (this is the bleaching process) and eventually die, since a major portion of a coral's nutrition comes from the photosynthetic products of the algae. Coral bleaching events of unprecedented frequency and global extent were reported in the 1980s and early 1990s (2, 5, 14–16, 22). Coral bleaching may be induced by a variety of environmental stimuli, including increased seawater temperature (17, 25), pollution (34), and ultraviolet radiation (13, 39). There have been speculations that large-scale bleaching episodes are linked to global warming. However, there is no clear evidence that environmental stress is the direct cause of coral bleaching.

Recently, we reported that bleaching of the coral *Oculina patagonica* (Fig. 1), present in the Mediterranean Sea, is the result of a bacterial infection (30, 31). The causative agent, *Vibrio* strain AK-1, was obtained in pure culture and shown to cause bleaching in controlled aquarium experiments. Furthermore, *Vibrio* strain AK-1-induced bleaching could be inhibited by antibiotics. The demonstration that *Vibrio* strain AK-1 caused bleaching of *O. patagonica* raised three fundamental questions: What is the mechanism of the infection, i.e., how does *Vibrio* strain AK-1 infection result in the expulsion of the zooxanthellae? How does environmental stress (e.g., temperature) influence the infection? And how general is the phe-

nomenon, i.e., are bacteria the causative agents of bleaching in other corals?

The present study was carried out in order to determine and compare the effects of seawater temperature on the bleaching of coral, induced by *Vibrio* strain AK-1, in aquaria and on the adhesion of *Vibrio* strain AK-1 to its coral host. Bacterial adhesion is often a first step in the colonization of host tissues and the subsequent establishment of infection in pathogenic systems (9). Adhesion can be a highly specific process (33, 38) involving the interaction of bacterial adhesins (generally proteins) and specific receptors (generally polysaccharides) on the external surface of the host. Since adhesion can be a prerequisite for successful infection, adhesins can be considered primary virulence factors. Once adhesion occurs, the bacteria can induce the expression of other virulence genes and cause the activation of host cell signalling pathways (9).

The present study of the adhesion of *Vibrio* strain AK-1 to *O. patagonica* provides information on the carbohydrate specificity of the process and on the effect of temperature on adhesion production. The data indicate that *Vibrio* strain AK-1 contains an adhesin that recognizes β-galactopyranosides on the coral surface. The adhesin is produced when *Vibrio* strain AK-1 is grown at 25°C but is not produced when the bacteria are grown at 16°C.

MATERIALS AND METHODS

Microorganisms. *Vibrio* strain AK-1 was isolated from a bleached coral as described previously (30, 31). The strain appears to be a new species of *Vibrio*, based on classical biochemical and physiological tests and 16S ribosomal-DNA sequence analysis, as well as its fatty-acid profile (data not presented). *Vibrio* strain AK-1 was maintained on MB agar (1.8% marine broth plus 0.9% NaCl solidified with 1.8% agar [both products of Difco Laboratories, Detroit, Mich.]). After streaking, the plates were incubated at 30°C for two days and then allowed to stand at room temperature for 1 week. A kanamycin-resistant mutant of *Vibrio*

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FIG. 1. Photograph of *O. patagonica* showing a bleached area (left) and a healthy area (right). Magnification, $\times 4$.

strain AK-1 was obtained by streaking cells onto MBK agar (MB agar containing 100 μ g of kanamycin per ml). A colony appearing after 2 days of incubation at 30°C was suspended in sterile seawater and restreaked on MBK agar. The kanamycin-resistant mutant *Vibrio* strain AK-2 was maintained on MBK agar as described above and used in all coral experiments described in this report. Several independent experiments indicated that with regard to bleaching of corals and adhesion to corals, strains AK-1 and AK-2 were identical. Strains LS-4 and LS-6 were isolated from Mediterranean Sea water from along the coast of Israel. These strains were also maintained on MB agar.

Collection and maintenance of the corals. Intact colonies of *O. patagonica* were collected from depths of 1 to 3 m along the Mediterranean coast of Israel. Seawater temperature at the time of collection was 25 to 26°C. Within 1 to 2 h of collection, each colony was split into several pieces and placed into 2-liter aerated aquaria containing filtered (0.45- μ m pore size) seawater that were maintained at 25°C. The aquaria were illuminated with a fluorescent lamp in cycles alternating 12 h of light with 12 h of darkness. Coral pieces were allowed to recover and regenerate for 15 days before the start of each experiment. If any piece failed to heal (complete coverage of the damaged skeleton by new tissue), it was discarded and not used in any experiment. For experiments at temperatures other than 25°C, the recovered corals were transferred to aquaria at the desired temperature and maintained for an additional 10 days.

Laboratory aquarium bleaching experiments. Ten microliters of seawater containing either 1.2×10^8 , 1.2×10^6 , 1.2×10^4 , or 1.2×10^2 cells of *Vibrio* strain AK-2 was placed directly on each of six healthy corals, and the corals were then put back in separate 2-liter aerated aquaria maintained at 16, 23, and 29°C. For a control, six corals were inoculated with 10 μ l of sterile medium and placed in separate aquaria at 16, 23, and 29°C. Prior to the experiment, the corals were acclimated to the different temperatures for 10 days. Percentage bleaching was determined qualitatively by visual observation.

Adhesion of bacteria to *O. patagonica*. Ten milliliters of a 24-h culture of *Vibrio* strain AK-2, grown at 25°C in MBK medium, was centrifuged at $10,000 \times g$ for 10 min at 25°C. The pellet was suspended in 10 ml of filter-sterilized seawater, and 0.1-ml samples were inoculated into 125-ml flasks containing 20 ml of sterile seawater plus a fragment of the regenerated (i.e., the coral had been removed from the aquarium and rinsed in sterile seawater) *O. patagonica* specimen (~ 1 cm² surface area). The flasks were incubated at $25 \pm 1^\circ\text{C}$ with gentle shaking at 40 rpm on a "Belly Dancer" (Stovall Life Sciences Inc., Greensboro, N.C.). Samples of the seawater were removed at timed intervals, diluted in seawater, and plated onto MBK agar. Two sets of control experiments were performed as described above. In the first, there were no added *Vibrio* strain AK-2 controls: the number of bacteria that eluted from the nonsterile fragments of coral and formed colonies on MBK agar was always less than 1% of the experimental values. In the second, there were no coral controls: there was a small but significant decrease in the numbers of CFU, even in the absence of coral, indicating that there was a slow binding of the bacteria to the walls of the flask. The results for this nonspecific binding are presented in Table 1. In order to calculate net adhesion, the value for the adhesion of the no-coral control was subtracted from the experimental value. Carbohydrates used to measure inhibition of adhesion were all products of Sigma Chemical Co., St. Louis, Mo.

The adhesion experiments with strains LS-4 and LS-6 were performed exactly as for *Vibrio* strain AK-2 except that the overnight culture was in MB medium and the diluted seawater was spread on MB agar. Values for the nonspecific adhesion of strains LS-4 and LS-6 to the flasks (no-coral controls) were subtracted from the values for the corresponding coral adhesion experiment to obtain net adhesion.

TABLE 1. Adhesion of *Vibrio* strain AK-2 to *O. patagonica*^a

Time (h)	Adhesion for flask containing ^b :		Net adhesion (%) ^c
	Coral	No coral	
2	45 $\pm$ 4.0	0 $\pm$ 1.0	45
6	80 $\pm$ 4.5	2 $\pm$ 0.5	78
8	86 $\pm$ 3.3	2 $\pm$ 0.7	84
12	91 $\pm$ 4.2	4 $\pm$ 1.1	87

^a To 125-ml flasks containing 20 ml of sterile seawater were added small fragments (~ 1 -cm² surface area) of *O. patagonica*. While the samples were being shaken gently at 25°C, 0.1 ml of an overnight culture of *Vibrio* strain AK-2 (5×10^8 organisms/ml) was inoculated into each flask. Viable counts in the seawater were determined on MBK agar after appropriate dilutions.

^b Adhesion (mean $\pm$ the standard error of the mean) was calculated from the decrease in bacteria in the seawater compared to the value at time zero (immediately after inoculation). The data are averages from five independent experiments.

^c Percent net adhesion to coral is the value obtained from the flask containing the coral minus the value for the no-coral control.

Desorption of *Vibrio* strain AK-2 from coral. Following adhesion experiments, the fragments of coral were removed from the flask and rinsed gently in 10 ml of sterile seawater. The corals were then placed in a fresh 10 ml of seawater containing 0.01% methyl- β -D-galactopyranoside and incubated for 5 min with gentle shaking. The resulting desorbed bacteria were diluted and plated onto MBK agar.

Sepharose bead experiments. The experiment to measure the adhesion of *Vibrio* strain AK-1 to Sepharose beads was performed in 1 ml of sterile seawater. Sepharose 4B-200 (45- to 165- μ m wet-bead diameter) and β -D-galactopyranoside-Sepharose (*p*-aminobenzyl-1-thio- β -D-galactopyranoside insolubilized on 4% beaded agarose, spacer of 12 atoms) beads were both products of Sigma Co. One milliliter of β -D-galactopyranoside-Sepharose beads binds 2 mg of β -galactosidase. After incubation of the cells with the suspended beads for 1 h with gentle shaking, the beads were allowed to settle for 10 min, and the supernatant fluids were diluted and plated onto MB agar.

RESULTS

Aquarium bleaching experiments. Table 2 documents the effect of inoculum size on bleaching at different temperatures. At 29°C, corals inoculated with only 120 bacteria showed 50 and 83% bleaching after 10 and 20 days, respectively. At 23°C, there was less bleaching than at 29°C, even when the initial inoculum was large. At 16°C, there was no bleaching at all, even with a large initial inoculum (1.2×10^8 cells). No bleaching occurred at any of the three temperatures when no bacteria

TABLE 2. Bleaching of the coral *O. patagonica* by *Vibrio* strain AK-1 as a function of inoculum size and temperature^a

Temperature (°C)	Bacterial concn (cells/10 liters)	Bleaching (%) after:	
		10 days	20 days
29	1.2×10^2	50	83
	1.2×10^4	67	83
	1.2×10^6	67	100
	0	0	0
23	1.2×10^4	17	50
	1.2×10^6	33	50
	1.2×10^8	33	67
	0	0	0
16	1.2×10^8	0	0
	0	0	0

^a For each temperature, 10 μ l of an overnight culture of *Vibrio* strain AK-1 was placed on each of the six healthy corals, and the corals were then returned to the aquarium.

were inoculated onto the coral. A typical example of a partially bleached coral is shown in Fig. 1.

Adhesion of *Vibrio* strain AK-1 to *O. patagonica*. An assay procedure was developed for examining the adhesion of *Vibrio* strain AK-1 to the coral *O. patagonica*. Small fragments (~1-cm² surface area) of coral that had recovered and regenerated in aquaria were placed in 250-ml flasks containing 20 ml of filter-sterilized seawater and shaken gently at 25°C. A kanamycin-resistant mutant of *Vibrio* strain AK-1 was used in all the binding studies to avoid enumerating any native bacteria that were associated with the nonsterile coral. Several experiments in aquaria indicated that the kanamycin-resistant mutant, *Vibrio* strain AK-2, behaved exactly like its parent with regard to bleaching *O. patagonica* (data not shown). Immediately after the inoculation of *Vibrio* strain AK-2 into the seawater, the numbers of viable bacteria in the water were determined (time zero) on media containing kanamycin. The numbers of viable bacteria in the water were then determined after different periods of incubation and compared to the values at time zero.

The data (averages from five independent binding experiments) are summarized in Table 1. In flasks containing coral, there was a rapid removal of *Vibrio* strain AK-2 from the water, with levels reaching 45, 80, 86, and 91% after 2, 6, 8, and 12 h, respectively. In the no-coral control, there was a slow adhesion to the glass walls of the flask, reaching 4% after 12 h. By subtracting the rate of nonspecific adhesion to the glass, the net adhesion to the corals was obtained. No significant difference was found when using *Vibrio* strain AK-1 or the kanamycin-resistant mutant *Vibrio* strain AK-2.

Strain specificity of *Vibrio* adhesion to *O. patagonica*. Adhesion of *Vibrio* strain AK-1 to the coral *O. patagonica* was specific for that strain and was not a general property of marine bacteria. Several gram-negative, motile rods were isolated from the tidal water surrounding the coral and tested for adhesion to *O. patagonica*. None of these bacteria showed any significant net adhesion. Results for a typical experiment are shown in Fig. 2.

Inhibition of adhesion of *Vibrio* strain AK-2 to *O. patagonica* by carbohydrates. Of the four monosaccharides tested, only D-galactose inhibited net adhesion of *Vibrio* strain AK-2 to *O. patagonica* (Table 3). The inhibition was stronger with methyl-β-D-galactopyranoside than with D-galactose or methyl-α-D-galactopyranoside. Even with 0.001% (50 μM) methyl-β-D-galactopyranoside, there was 94% inhibition of *Vibrio* strain AK-2 adhesion to *O. patagonica*. The methyl-α-D-galactopyranoside was a better inhibitor than D-galactose at the lower concentrations tested. It should be pointed out that methyl-β-D-galactopyranoside cannot serve as a carbon or energy source for *Vibrio* strain AK-1.

Desorption of *Vibrio* strain AK-2 from *O. patagonica*. The experiments whose results are summarized in Tables 1 and 3 measured net adhesion of the bacteria to the coral by comparing the decrease of bacteria in seawater in a flask containing the coral with that for a corresponding no-coral control. To measure directly the bacteria bound to *O. patagonica*, the coral fragments were rinsed and the bound bacteria were desorbed with 0.01% methyl-β-D-galactopyranoside (Table 4). The treatment caused no disruption of the soft tissue of the coral or release of the endosymbiotic zooxanthellae. After 6 h, 2.5×10^7 *Vibrio* strain AK-2 organisms could be recovered from the coral, compared to only 4×10^4 when the seawater contained 0.01% methyl-β-D-galactopyranoside. Since 6.0×10^7 *Vibrio* strain AK-2 organisms were inoculated into the flask, the recovered bacteria represented only 42% of the input, compared to 78% net adhesion as measured by loss from the water. Increasing the incubation time to 12 h resulted in a slightly

FIG. 2. Kinetics of adhesion of *Vibrio* strain AK-2 (●), strain LS-4 (○), and strain LS-6 (▲) to *O. patagonica*. The experiment was performed as described in Table 1 and in Materials and Methods.

higher number of *Vibrio* strain AK-2 organisms recovered from the coral, reaching 3.1×10^7 bacteria, or 52% of the number of cells input. Less than 0.2% of the input *Vibrio* strain AK-2 organisms were recovered from the coral after 6 or 12 h when methyl-β-D-galactopyranoside was used as an inhibitor of adhesion.

The effect of temperature on adhesion of *Vibrio* strain AK-1 to *O. patagonica*. As demonstrated above, *Vibrio* strain AK-1 infects and causes bleaching of *O. patagonica* at 23 and 29°C

TABLE 3. Inhibition of adhesion of *Vibrio* strain AK-2 to *O. patagonica* by carbohydrates^a

Carbohydrate	Concn (%)	Net adhesion (%)	Inhibition (%)
None		81	
L-Arabinose	0.1	79	2
D-Glucosamine	0.1	75	7
X-Xylose	0.1	87	0
D-Galactose	0.001	27	65
	0.01	20	77
	0.1	24	72
Methyl-α-D-galactopyranoside	0.001	27	65
	0.01	10	88
	0.1	14	82
Methyl-β-D-galactopyranoside	0.001	6	94
	0.01	0	100
	0.1	0	100

^a The experiment was performed in duplicate as described in Table 2. The percent net adhesion was determined after 6 h by subtracting the value for the adhesion of bacteria to the walls of the flask (control) from the value for the adhesion of bacteria to the flask containing the coral.

TABLE 4. Adhesion of *Vibrio* strain AK-2 to and desorption from *O. patagonica*^a

Components	Time (h)	Net adhesion (%) ^b	Desorption ^c	
			(Total CFU)	(% Input)
Coral plus bacteria	6	78	2.5×10^7	42
	12	80	3.1×10^7	52
Coral plus bacteria plus Me- β -D-gal ^d	6	0	4×10^4	0.07
	12	10	8×10^4	0.13

^a The experiment was performed as described in Table 2, with an initial inoculum of 6.0×10^7 *Vibrio* strain AK-2 organisms per 20 ml.

^b Adhesion was measured by determining the number of viable *Vibrio* strain AK-2 organisms remaining in the seawater.

^c Desorption was measured by removing the coral, rinsing it in sterile seawater, and then incubating it in 10 ml of 0.01% methyl- β -D-galactopyranoside in sterile seawater in order to desorb bacteria bound to the coral tissue.

^d The concentration of methyl- β -D-galactopyranoside (Me- β -D-gal) was 0.01%.

(Table 2) but not at 16°C. The possibility that adhesion of the pathogen to its host was a function of temperature was therefore examined. As seen in Table 5, when the bacteria and coral were both grown at 16°C and the adhesion experiment was conducted at 16°C, there was insignificant adhesion compared to the situation with the 25°C control. The critical parameter appears to be the temperature of bacterial growth, because bacteria grown at 16°C did not adhere to corals grown at 25°C, whereas bacteria grown at 25°C adhered to corals grown at 16°C. Since adhesion of bacteria grown at 25°C was somewhat better to corals grown at 25 than those grown at 16°C, it is possible that the corals also play a part in the effect of temperature on adhesion.

Adhesion of *Vibrio* strain AK-1 to β -D-galactopyranoside-Sepharose beads as a function of bacterial growth temperature. As seen in Table 6, *Vibrio* strain AK-1, grown at 25°C, adhered efficiently to Sepharose beads containing bound β -D-galactopyranoside. The use of 1 or 5 μ l of the bead suspension resulted in >98% of the cells adhering to the beads. When the cells were grown at 16°C, 7.5 and 17% adhesion occurred to 1 and 5 μ l of the beads, respectively. Controls, with Sepharose beads that did not contain bound β -D-galactopyranoside, showed 7 to 9% adhesion for cells grown at 16 or 25°C.

TABLE 5. Adhesion of *Vibrio* strain AK-2 to *O. patagonica* as a function of temperature^a

Temp of process (°C)			Net adhesion (%)
Bacterial growth	Coral growth	Adhesion expt	
16	16	16	7
16	25	16	0
16	25	25	0
25	25	25	79
25	16	25	47
25	16	16	53

^a The experiment was performed as described in Table 2, except that the bacteria were grown overnight at either 16 or 25°C, the corals were maintained in an aquarium at 16 or 25°C for at least 1 week, and the incubation temperature during the 6-h adhesion test was varied.

TABLE 6. Adhesion of *Vibrio* strain AK-1 to Sepharose beads

Vol of beads (μl) ^a	Temp of bacterial growth (°C)	Cells/ml		Adhesion (%)
		Input	After 1 h	
Sephareose				
100	25	5.1 × 10 ⁷	4.7 × 10 ⁷	7.8
10	25	5.8 × 10 ⁷	5.3 × 10 ⁷	8.6
10	16	5.4 × 10 ⁷	5.0 × 10 ⁷	7.4
Sephareose-β-D-galactopyranoside				
5	25	4.8 × 10 ⁷	8.7 × 10 ⁵	98.2
1	25	5.7 × 10 ⁷	9.1 × 10 ⁵	98.4
5	16	5.3 × 10 ⁷	4.4 × 10 ⁷	17.0
1	16	5.3 × 10 ⁷	4.9 × 10 ⁷	7.5

^a The beads are described in Materials and Methods.

DISCUSSION

There have been a large number of studies that demonstrate a correlation between increased seawater temperature and coral bleaching. Furthermore, it has been shown experimentally in aquaria that raising the water temperature can cause bleaching of corals (25). This has led to the speculation that increased seawater temperature, resulting from global warming or El Nino events, is the direct cause of coral bleaching (14). For example, it has been suggested that the increased temperature could induce the coral to produce heat shock proteins which might cause bleaching (20). There are a number of weak points in this argument. First, the correlation is not one of cause and effect. In fact, Oliver (35) and Fisk and Done (10) have shown that extensive bleaching in the Great Barrier Reef during the summer of 1982 was not associated with any major sea surface temperature increases. Second, several authors have reported on the patchy spatial distribution and spreading nature of coral bleaching (10, 25, 32, 35). Hayes and Bush (19) have suggested that the random mosaic pattern of bleaching within a coral colony is difficult to attribute solely to environmental variables such as seawater temperature. Segments of the colony, rather than the entire colony, respond to stress by expelling zooxanthellae. There is no consistency to the sizes of the bleached zones or to their locations within the colony. Observations of progressive recovery in the coral colony suggest that bleaching may begin within a small zone and spread from there into adjacent areas of the colony (19). The progression of observable changes that take place during coral bleaching is reminiscent of that of developing microbial biofilms on other biological tissues (23) or inorganic surfaces (24). Third, the fact that raising the temperature in an aquarium can cause bleaching (25) does not demonstrate that the temperature increase is the direct cause of the bleaching. In the present study, it was shown that increased temperature allows for more efficient infection by *Vibrio* strain AK-1 of its host and for the subsequent bleaching. In general, climate-related increases in sea surface temperature can lead to a higher incidence of water-borne infections and toxin-related illnesses, such as cholera (8, 36).

The temperature dependence of bacterium-induced bleaching of *O. patagonica* is clearly demonstrated in this study. The temperature was a more critical factor for initiating the infection than the inoculum size. As few as 10^6 bacteria caused rapid bleaching at 29°C, whereas 10^8 bacteria failed to cause bleaching at 16°C. It should be pointed out that the *O. patagonica* samples used in these experiments were healthy corals

taken from the sea in the summer and thus that they probably were not contaminated with *Vibrio* strain AK-1.

The data presented here demonstrate that the coral-bleaching pathogen *Vibrio* strain AK-1 adheres to its host coral, *O. patagonica*. The adhesion is blocked by D-galactose and by very low concentrations of methyl- β -D-galactopyranoside. Inhibition of adhesion by β -D-galactosides is characteristic of bacteria containing type P fimbriae, such as certain uropathogenic *Escherichia coli* (33). The classical strain of *Vibrio cholerae* attaches to L-fucose receptors of the free brush border membranes of epithelial cells (27).

Previous studies have demonstrated the adhesion of different strains of *Vibrio* to surfaces. Belas and Colwell (3) studied the kinetics of adsorption of *Vibrio* to chitin and concluded that both polar and lateral flagella contribute to binding. In the case of pathogenic bacteria, there have been conflicting data on the role of flagella in attachment (1, 18, 21, 26, 37). The adhesion of selected fish-pathogenic *Vibrio* strains to the skin mucus of fish has also been studied (4, 29). Temperature and salinity played important roles in adhesion to fish mucus.

The adhesion of *Vibrio* strain AK-1 to coral and inhibition of the adhesion by methyl- β -D-galactopyranoside were also demonstrated by desorbing the bound bacteria from the coral with 0.01% methyl- β -D-galactopyranoside (Table 4). Adhesion values obtained from desorption data were significantly lower than values determined by removal from seawater. There are several possible explanations for this difference. First, it may be that all of the bound bacteria were not desorbed by the procedure employed. Second, some of the bound *Vibrio* strain AK-1 organisms may have been killed by the coral. In this regard, it is known that fish mucus has strong antibacterial activity (11), including activity against *Vibrio anguillarum* (40). Also, it has recently been shown that scleractinian corals exhibit antibacterial activity against marine *Vibrio* strains (28). Third, electron-microscopic observations of *Vibrio* strain AK-1 on the surfaces of corals indicate that the bacteria are present in large aggregates (30, 31). It is possible that the desorption conditions did not completely break up the aggregates. Any of these explanations would result in an underestimation of the number of bound bacteria.

The role of β -D-galactopyranoside residues on the coral surface, acting as receptors for the *Vibrio* strain AK-1 adhesins, was confirmed by the use of Sepharose beads containing bound β -D-galactopyranoside. The fact that *Vibrio* strain AK-1 grown at 16°C failed to adhere to the galactose-containing beads, whereas bacteria grown at 25°C adhered avidly, further supports the conclusion that the *Vibrio* strain AK-1 adhesin recognizes β -D-galactopyranoside residues and that production of the adhesin is temperature regulated. The strong binding of *Vibrio* strain AK-1 grown at 25°C to the derivatized beads should allow for the ready isolation of adhesin-defective mutants as well as of deregulated mutants, e.g., bacteria that produce the adhesin at 16°C.

Probably the most significant ecological aspect of this study was the discovery that the temperature of bacterial growth is critical for the adhesion process. It has generally been assumed that coral bleaching brought about by elevated seawater temperatures (6, 12, 14, 15, 22, 25) is due to changes in coral physiology, such as the possible production of heat shock proteins (20). The data presented here suggest an alternative hypothesis, namely that the elevated temperature causes the coral-bleaching bacterium to be more virulent. This latter hypothesis is supported by a considerable body of information indicating that many virulence genes of pathogenic bacteria are transcribed more efficiently at higher growth temperatures. In this regard, it has recently been reported that induction of

cascades of virulence factors occurs following P-pili mediated binding of *E. coli* to its host cell receptor (41). It will now be interesting to examine if other virulence factors are expressed after *Vibrio* strain AK-1 adheres to *O. patagonica* (and coated beads) at elevated temperatures.

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