Environmental Microbiology (2003) **5**(4), 250–255

The marine fireworm *Hermodice carunculata* is a winter reservoir and spring-summer vector for the coral-bleaching pathogen *Vibrio shiloi*

Meir Sussman,[1,2] Yossi Loya,[1] Maoz Fine[1] and Eugene Rosenberg[2]*

[1]*Department of Zoology, and* [2]*Molecular Microbiology and Biotechnology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Ramat Aviv, Israel 69978.*

Summary

***Vibrio shiloi*, the causative agent of bleaching of the coral *Oculina patagonica* in the Mediterranean Sea, is present in all bleached *O. patagonica* corals in the summer (25–30°C), but can be not detected in the coral during the winter (16–20°C). Furthermore, the pathogen can not survive in *O. patagonica* at temperatures below 20°C. Using fluorescence *in situ* hybridization (FISH) with a *V. shiloi*-specific oligonucleotide probe, we found that the marine fireworm *Hermodice caranculata* is a winter reservoir for *V. shiloi*. Worms taken directly from the sea during the winter contained ~10^8 *V. shiloi* per worm by FISH analysis. However, colony-forming units (cfu) revealed only $4.1–18.3 \times 10^4$ *V. shiloi* per worm, indicating that ~99.9% of them were in the viable-but-not-culturable (VBNC) state. When worms were infected with *V. shiloi*, most of the bacteria adhered to the worm within 24 h and then penetrated into epidermal cells. By 48 h, less than 10^{-4} of the intact *V. shiloi* in the worm gave rise to colonies, suggesting that they differentiated inside the worm into the VBNC state. When worms infected with *V. shiloi* were placed in aquaria containing *O. patagonica*, all of the corals showed small patches of bleached tissue in 7–10 days and total bleaching in 17 days. This is the first report of a reservoir and vector for a coral disease.**

Introduction

Coral bleaching is the disruption of symbioses between coral hosts and endosymbiotic algae (zooxanthellae). Each summer for the last 10 years, when surface seawater temperatures rose to a maximum of 30–31°C in the Mediterranean Sea off the coast of Israel, 80–90% of the *Oculina patagonica* coral colonies underwent bleaching (Israely *et al*., 2001). During the winter when seawater temperatures fell to a minimum of 16°C, the corals recovered. The causative agent of this disease is *Vibrio shiloi* (Kushmaro *et al*., 1996; 1997). Temperature is a critical factor in determining the outcome of the *V. shiloi* infection of *O. patagonica* because several of the bacterial virulence factors are temperature regulated (Rosenberg and Ben-Haim, 2002). Using specific anti-*V. shiloi* antibodies it was shown that *V. shiloi* was present in the tissue of all bleached corals in the summer, but could not be detected in healthy or bleached corals in the winter (Israely *et al*., 2001). Laboratory aquaria experiments indicated that when corals were infected with *V. shiloi* at 28°C, and then shifted slowly to their winter *in situ* temperature (16°C), the bacteria died and lysed. These data indicate that bleaching of *O. patagonica* in the Mediterranean Sea requires a fresh infection each spring, rather than the activation of dormant intracellular bacteria. Here we show that the marine fireworm, the amphinomid polychaeta *Hermodice carunculata,* is a winter reservoir of *V. shiloi* that can serve as a vector for the transmission of the bleaching disease in the spring-summer when temperatures rise.

Received 28 October, 2002; accepted 7 January, 2002. *For correspondence. E-mail eros@post.tau.ac.il; Tel. (+972) 3 640 9838; Fax: (+972) 30–642 9377.

Results

Presence of Vibrio shiloi *inside* Hermodice caranculata

Using the specific *V. shiloi* oligonucleotide probe MS6 (FISH analysis), several samples of seawater, sediments and marine organisms were screened for the presence of *V. shiloi* during the winter when it could not be detected in its summer host *O. patagonica*. Although *V. shiloi* was detected sporadically and in low numbers in water and sediment samples, it appeared to be present in high concentrations in all fireworms taken directly from the sea. A photograph of *H. carunculata* is shown in Fig. 1. Tissue slices of FISH-stained worms showed that *V. shiloi* was present primarily in the epidermis of the worms, often in

Fig. 1. Photograph of the fireworm *H. carunculata* feeding on a coral colony.

large clumps (Fig. 2). The morphology of the worm-associated *V. shiloi* was similar to that found in corals (Banin *et al*., 2001). Controls with a non-complimentary fluorescent probe were negative. Table 1 summarizes an experiment in which five worms taken from different locations and depths off the Mediterranean Coast of Israel during the winter 2001/2002 were homogenized and analysed for *V. shiloi* by colony-forming units (cfu) and FISH analysis. By FISH analysis all five worms contained high numbers of *V. shiloi* ($0.6–2.9 \times 10^8$). When corrected for worm length, the average and standard error of the mean for the five worms was $1.6 \pm 0.2 \times 10^7$ *V. shiloi* per cm. These values refer to the average over the entire worm, as the worm was crushed before analysis. Actually, the local concentrations were much higher, as seen in Fig. 2.

The *V. shiloi* cfu values for each of the five worms were more than 1000-fold lower than the FISH values, varying from 4.1 to 18.3×10^4 cfu per worm. Correcting for the different lengths of the worms yields an average and standard error of the mean of $4.8 \pm 1.1 \times 10^3$ cfu/cm. The ratio of cfu to FISH value varied from 1.3 to 6.8×10^{-4}, with an average of 4.8×10^{-4}. Thus, *V. shiloi* was present in high and rather similar concentrations in all fireworms taken from the sea during the winter and more than 99.9% of them were in the viable-but-not-culturable (VBNC) state.

Adhesion of Vibrio shiloi *into* Hermodice caranculata

Inoculation of *V. shiloi* into flasks containing *H. caranculata* resulted in a time-dependent decrease of the bacteria in the seawater (Fig. 3). After 24 h and 36 h, 23% and less than 5%, respectively, of the inoculated *V. shiloi* were detected in the seawater. In control flasks, containing no worm, the concentration of *V. shiloi* remained constant for at least 48 h. The worm by itself released very few bacteria into the water.

Direct determination of the number of *V. shiloi* associated with the worm following the adhesion experiment was performed by crushing the worms in sterile seawater and (a) plating dilutions on TCBS agar and (b) by direct microscopic counts on the homogenized worm using the specific *V. shiloi* fluorescent oligonucleotide probe MS6 (FISH analysis). As summarized in Table 2, after 24 h and 48 h, the number of colony-forming units (cfu) in worms inoculated with the bacterium was 2.9×10^7 and 1.5×10^4 respectively. As the total number of *V. shiloi* inoculated in each flask was 1.2×10^9, only a small fraction of bacteria removed from the water was recovered as cfu associated with the worm. Furthermore, the number of colony-forming *V. shiloi* recovered from the worm decreased ~2000 times from 24 h to 48 h after inoculation. On the other hand, FISH analysis which measures total intact *V. shiloi* yielded 7.0×10^8 and 3.5×10^8 bacteria after 24 h

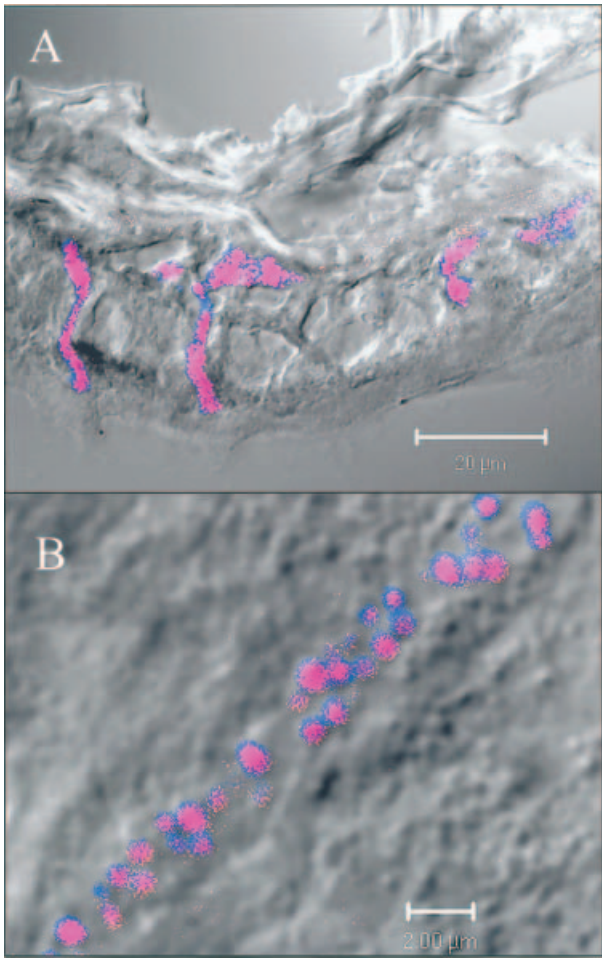

Fig. 2. Presence of *V. shiloi* in the fireworm *H. carunculata*. Using a *V. shiloi*-specific fluorescent oligonucleotide probe, the bacteria appear purple by fluorescence *in situ* hybridization (FISH) of tissue sections. A, low magnification; B, higher magnification.

Table 1. Presence of *Vibrio shiloi* in *Hermodice caranculata*.

	Vibrio shiloi per worm		
Worm length (cm)	cfu	FISH	Ratio (cfu/FISH)
6	4.1×10^4	1.1×10^8	3.7×10^{-4}
5	4.1×10^4	0.7×10^8	5.8×10^{-4}
13	18.3×10^4	2.9×10^8	6.3×10^{-4}
7	7.8×10^4	0.6×10^8	1.3×10^{-4}
7	8.8×10^4	1.3×10^8	6.8×10^{-4}

Worms were collected from different depths during the winter 2001/2001, homogenized and analysed for colony-forming units (cfu) and total *V. shiloi* (FISH) as described in *Experimental procedures*.

and 48 h respectively. Thus, 58% of the *V. shiloi* inoculated into the flask were found in the worm after 24 h. The fact that after 48 h, less than 10^{-4} of the intact *V. shiloi* in the worm gave rise to colonies on TCBS agar suggests that they differentiated inside the worm into a VBNC state.

Electron micrographs of thin sections of *H. caranculata* incubated with *V. shiloi* for 24 h shows the bacteria inside epidermal cells of the worm (Fig. 4). The bacteria were also concentrated inside invaginations of the outer membrane of *H. caranculata*, suggesting a possible mechanism of uptake. The fact that the intercellular bacteria were *V. shiloi* was verified by FISH analysis of thin sections of the infected worm. The bacteria appear to enter *H. caranculata* in the form of strings perpendicular to the long axis of the worm.

Vibrio shiloi-*infected* Hermodice caranculata *causes bleaching of Oculina patagonica*

To test if *H. caranculata* containing *V. shiloi* can cause bleaching of the coral *O. patagonica*, laboratory infected worms were placed in aquaria that contained the corals (Table 3). All six corals (aquaria B and C) that were exposed to *V. shiloi*-infected worms were totally bleached within 6 weeks. Control corals (aquarium A) that were not exposed to *H. caranculata* showed no signs of bleaching for at least 3 months. Out of five corals exposed to a non-infected worm (aquarium D) only one bleached, whereas the rest of the corals remained healthy. The two corals that were exposed to a worm that was infected with *Vibrio mediterranei* (the closest known relative of *V. shiloi*) did not bleach or exhibit any signs of disease.

Photographs of *H. caranculata*-induced bleaching of *O. patagonica* are shown in Fig. 5. Approximately 7–10 days after exposure to the infected worm, corals showed small patches of bleached tissue in the middle of the coral, surrounded by healthy tissue (Fig. 5A). After 17 days most of the corals were totally bleached (Fig. 5B), and macroalgae began to appear on the bleached tissue. Bleaching can easily be identified by the transparent coral tentacles.

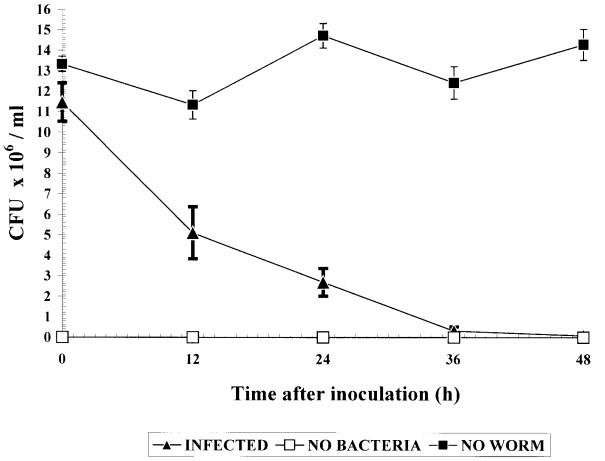

Fig. 3. Adhesion of *Vibrio shiloi* to *Hermodice caranculata*. A 24 h culture of *V. shiloi*, grown at 30°C with aeration in MB medium was centrifuged, the cell pellet washed and resuspended in sterile seawater and then inoculated into flasks containing one *H. caranculata* in 150 ml sterile seawater. Flasks were incubated at 28°C with gentle shaking. At the indicated times, water samples were removed from the flasks, diluted and plated on TCBS agar. Colony-forming units (cfu) ± SEM are plotted as a function of time for flasks containing the worm and bacteria (▲), bacteria alone (■) and worm alone (□).

Discussion

Although the data presented here demonstrate that *V. shiloi* is present in *H. caranculata* and that worms infected with *V. shiloi* can transmit the pathogen to *O. patagonica* and cause bleaching, no information is available regarding the frequency of this mode of transmission. During the summer, 80–90% of the *O. patagonica* colonies undergo bleaching. Based on field observations it seems unlikely that *V. shiloi*-infected worms come into direct contact with all of the bleached colonies. As a working hypothesis we suggest that the worm serves as a major reservoir for *V. shiloi* during the winter and then in the spring when water temperatures warm and the worm begins to feed on *O. patagonica*, it transmits the pathogen to a few corals. The bacteria multiply intracellularly in the coral (Banin *et al*.,

Table 2. Uptake of *Vibrio shiloi* into *Hermodice caranculata*..

	Vibrio shiloi per worm		
Incubation time (h)	cfu	FISH	Ratio (cfu/FISH)
0	$>10^3$	$0.5 \pm 0.2 \times 10^8$	$>2 \times 10^5$
24	2.9×10^7	$7.0 \pm 1.6 \times 10^8$	4.1×10^{-2}
48	1.5×10^4	$3.5 \pm 1.0 \times 10^8$	4.3×10^{-5}

The experimental protocol is exactly as described in Fig. 3 except that at timed intervals, the infected worm was removed from the flask, rinsed in sterile seawater and then crushed. The number of *V. shiloi* on or in the worm was determined by plating on TCBS agar for colony-forming units (cfu) and by FISH analysis for total *V. shiloi*.

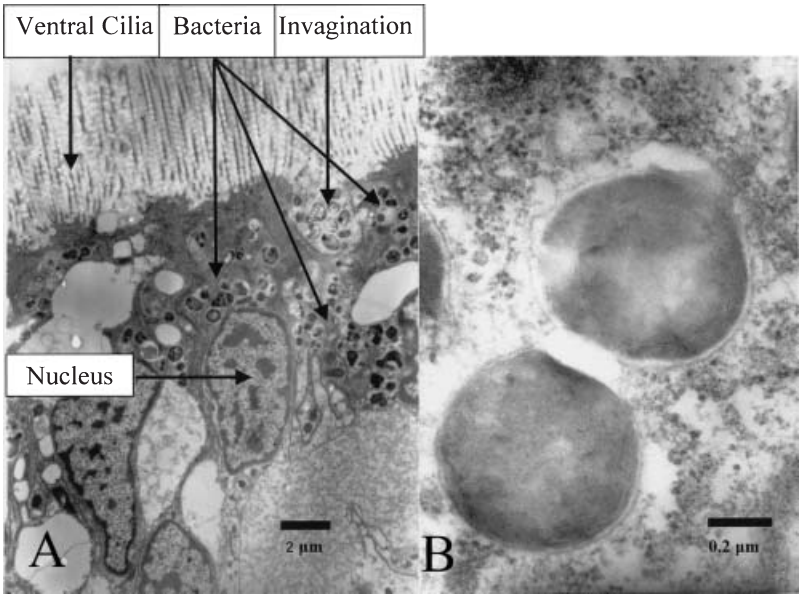

Fig. 4. Electron micrograph of sectioned *H. carunculata* 24 h after the worm was infected with *V. shiloi*. At low magnification (A), the bacteria can be seen inside worm cells and in an invagination of the outer membrane. At higher magnification (B), the morphology of the bacteria can be observed.

Table 3. Coral bleaching by *Vibrio shiloi*-infected *Hermodice caranculata.*

Aquarium	*O. patagonica* (n)	*H. caranculata*	Bleaching after 6 weeks
A	10	None	0/10
B	3	*V. shiloi*-infected	3/3
C	3	*V. shiloi*-infected	3/3
D	6	Non-infected	1/5
E	2	*V. mediterranei*-infected	0/2

Worms were infected in flasks as described in Fig. 3 with 5×10^7 *Vibrio shiloi* or 1×10^8 *Vibrio mediterranei*. After 24 h the worms were removed from the flasks, rinsed with sterile seawater and then placed in aquaria containing the corals at 28°C. Except for control aquarium A, each aquarium received one infected or non-infected worm.

2000), produce toxins (Ben-Haim *et al.*, 1999; Banin *et al.*, 2001) and bleach the infected coral. The bleaching is the result of both destruction of zooxanthellae and release of intact algae. We suggest that *V. shiloi* associated with the released zooxanthellae are taken up by healthy corals, causing mass bleaching in the summer.

Vibrio shiloi was present in the VBNC state inside *H. caranculata* both with fireworms taken directly from the sea and in laboratory infection experiments. These data are similar to what have been reported for *V. shiloi* infections of *O. patagonica* (Banin *et al.*, 2000). In the latter case it was demonstrated that *V. shiloi* in the VBNC state was infectious (Israely *et al.*, 2001). Bacteria in the VBNC state adhered to *O. patagonica*, penetrated into the exoderm and multiplied intracellularly. Although it was not demonstrated directly in the present study, it is likely that VBNC *V. shiloi* inside the fireworm was transmitted from the worm to the coral during feeding and was responsible for the infection and subsequent bleaching.

Transmission of *V. shiloi* from the fireworm to *O. patagonica* depends upon direct contact. There have been

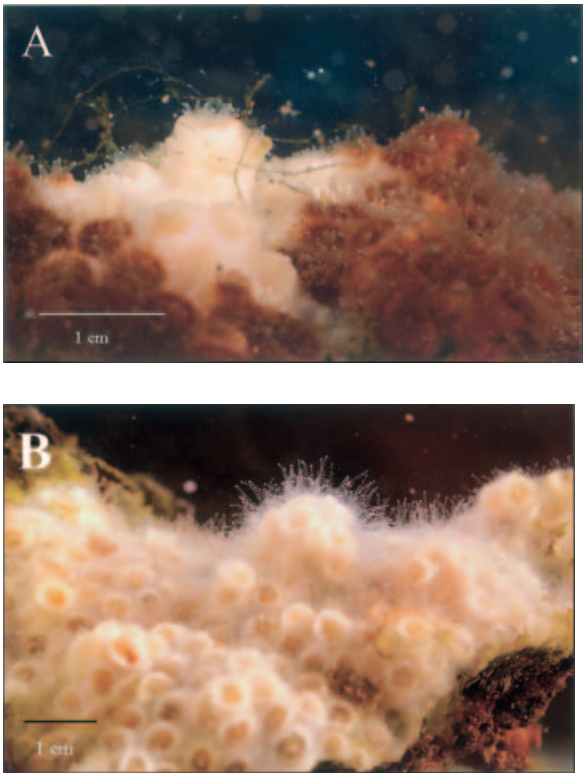

Fig. 5. Bleaching of the coral *O. patagonica* by *H. carunculata* that was infected in the laboratory with *V. shiloi*. Coral bleaching after (A) 7 days and (B) 17 days of contact between the worm and the coral.

several reports of *H. caranculata* feeding on corals (Rattenbury-Marsden, 1963; Lewis and Crooks, 1996; Witman, 1998). Along the Mediterranean coast it has been observed that *H. caranculata* feeds on *O. patagonica* only at night, both in shallow and deep water (Fine *et al*., 2002). During the day, the worm hides under rocks and in crevices. Knowledge about reservoirs and modes of transmission has proven useful in the past for developing technologies for controlling the spread of disease. For example, the presence of *Vibrio cholerae* in marine cocepods (Rozhak and Colwell, 1987; Colwell, 1996) has been implicated in the spread of cholera, and simple filtration of drinking water through a nylon net or sari material removed the attached *V. cholerae* (Huq *et al*., 1996).

Experimental procedures

Microorganism and growth media

Vibrio shiloi, a new species of *Vibrio* (Kushmaro *et al*., 2001), was isolated from a bleached coral as described previously (Kushmaro *et al*., 1996). The strain was maintained on MB agar (1.8% marine broth plus 0.9% NaCl solidified with 1.8% agar, both products of Difco Laboratory). After being streaked onto MB agar, the cultures were incubated at 30°C for 2 days and then allowed to stand at room temperature for 1 week. TCBS agar (Difco MA2216), a selective medium for *Vibrio*, was used periodically to confirm the purity of the strain.

Collection and maintenance of Oculina patagonica

For infection experiments, colony fragments of *O. patagonica* were collected during the winter when seawater temperatures were below 20°C, from depths of 0.5–1.5 m along the Mediterranean coastline of Israel. Within 1 h of collection, the coral fragments were placed in aerated aquaria containing filtered seawater at the ambient seawater temperature. The aerated aquaria were illuminated with a fluorescent lamp at 12 h light:12 h dark intervals. Coral pieces were allowed to recover, regenerate and acclimatize to 28°C for 3–4 weeks before the start of each experiment. If any piece failed to heal (complete cover of damaged skeleton by new tissue), it was discarded and not used in any experiment.

Collection and maintenance of Hermodice caranculata

Fireworms (*Hermodice caranculata*) were collected either by SCUBA at night at a depth of 30 m, 2.5 km off the coast (50 km north of Tel Aviv) or from a protected harbour (49 km north of Tel Aviv). Worms were kept in 10-litre tanks in the dark and fed periodically with brine shrimp. Before each experiment, worms were rinsed with sterile seawater and placed in separate containers containing sterile seawater. All worms used in the experiments were 5–13 cm in length with a diameter of 1 cm.

Enumeration of Vibrio shiloi

Two methods were used to determine the concentration of *V. shiloi* inside the fireworm: (i) colony-forming units (cfu) and (ii) fluorescence *in situ* hybridization (FISH) analysis (Amann *et al*., 1997). In both cases worms were first rinsed in sterile seawater, crushed in 10 ml sterile seawater using a mortar and pestle, and then vortexed in a 50 ml tube for 1 min. For cfu, triplicate samples of appropriate dilutions were spread on TCBS agar. *Vibrio shiloi* has a characteristic colony morphology on TCBS agar (yellow, non-mucoid, serrated edges, 2–3 mm diameter after 48 h). Confirmation that the colonies were *V. shiloi* was obtained by checking cells by FISH. The standard errors for all determinations of cfu were less than 10%.

The second method (FISH) involved fixing samples of the crushed worm in 3% paraformaldehyde/PBS (24 g NaCl, 0.6 g KCl, 4.32 g Na_2HPO_4 and 0.72 g KH_2PO_4 per l) for 8 h at 4°C. The samples were then centrifuged for 5 min at 10 000 *g*, and the sediment suspended in PBS and centrifuged again. The sediment was washed an additional time in PBS before suspending the sediment in PBS/ethanol (1/1, v/v). Samples were stored at −20°C. Hybridization was performed as described previously (Pernthaler *et al*., 1998). Multiwell Teflon-coated slides were treated at 60°C with a solution of 0.01% $CrK(SO4)_2$ and 0.1% gelatin for 2 min. Fixed samples (20 μl) were then transferred to the 6 mm wells on the slide and placed at 60°C until dry. Slides were then passed sequentially through 50%, 80% and 100% ethanol, and air-dried. Hybridization was carried out by adding to each well 8 μl hybridization buffer [0.9 M NaCl, 20 mM Tris-HCl buffer, pH 7.2, 0.01% sodium dodecyl sulphate (SDS) and 50% (v/v) formamide] and 1 μl (30 ng μ^{-1}l) fluorescently labelled oligonucleotide. Two oligonucleotide probes, synthesized by MWG Biotech, Ebersberg, Germany, were used in this study: the general bacterial probe EUB 338 (5′-GCT GCCTCCCGTAGGAGT-3′, modified at the 5′ end with the indocarbocyanine dye cy5) and a *V. shiloi* specific probe MS6 (5′-AGTTTTACATTTGCGACC-3′, modified at the 5′ end with cy3). After allowing for hybridization at 46°C for 90 min, the slides were rinsed with prewarmed (48°C) washing buffer (20 mM Tris HCl, 5 mM EDTA, 0.01% SDS, 28 mM NaCl) and air-dried. Finally, slides were stained with 1 μg ml^{-1} DAPI solution (Sigma), St Louis, MO (USA) and dried at 60°C. Beginning with the hybridization, all steps were performed in the dark. Slides were viewed by a Zeiss Confocal Fluorescent microscope using AF87 (Citifluor) as an antifadant. Image analysis was performed with the Zeiss LSM 5 Image Browser software. The total number of *V. shiloi* per worm was calculated from the number of bacteria that hybridized with both probes (EUB 338 and MS6) on the slide multiplied by the dilution factor. Each slide contained 10 wells and in each well at least five fields were counted. Using the stringent hybridization condition (50% formamide), the closest known relative of *V. shiloi*, *Vibrio mediterranei* (ATCC 43341) failed to hybridize with the *V. shiloi* specific probe. Also, controls performed as described above on crushed worms using the non-complimentary fluorescent oligonucleotide probe Non-EUB 338 (5′-ACTCCTACGGGAGGCAGC-3′), yielded no signal.

Fluorescence in situ *hybridization (FISH) of tissue sections*

Complete worms were fixed in 3.7% filter sterilized formalde-

hyde in seawater for 7 h at 4°C and then rinsed twice with PBS, according to Manz *et al*. (2000). The fixed worms were stored at −20°C in a 1:1 (v:v) solution of PBS and 95% ethanol. Tissue samples were treated with protect-RNA (Sigma). Samples were embedded in paraffin and 14 μm thick tissue sections were prepared, using a sterile blade on a microtome. The sections were transferred to polylysine-coated slides and dried overnight. Prior to hybridization, paraffin was removed with xylene. The hybridization procedure was as described above except that 16 μl of hybridization buffer and 2 μl each of the oligonucleotide probes were used. Rinsing, DAPI staining and microscopy were as described above.

Electron microscopy

Intact worms were fixed in 2.5% glutaraldehyde, dehydrated in a graded series of ethyl alcohol and embedded in Epon. Sections stained with uranyl and lead citrate were viewed with a JEOL 1200 EX electron miscroscope.

Acknowledgements

We thank A. Barbul, E. M. Fine, Y. Delarea, Y. Aloma, A. Shoob and A. Lehner for technical assistance. This study was supported by the Israel Center for Emerging Diseases and the Pasha Gol Chair for Applied Microbiology.

References

Amann, R., Glockner, F.O., and Neef, A. (1997) Modern methods in subsurface microbiology: *in situ* identification of microorganisms with nucleic acid probe. *FEMS Microbiol Rev* **20:** 191–200.

Banin, E., Israely, T., Kushmaro, A., Loya, Y., Orr, E., and Rosenberg, E. (2000) Penetration of the coral-bleaching bacterium *Vibrio shiloi* into *Oculina patagonica*. *Appl Environ Microbiol* **66:** 3031–3036.

Banin, E., Khare, S.K., Naider, F., and Rosenberg, E. (2001) A proline-rich peptide from the coral pathogen *Vibrio shiloi* that inhibits photosynthesis of zooxanthellae. *Appl Environ Microbiol* **67:** 1536–1541.

Ben-Haim, Y., Banin, E., Kushmaro, A., Loya, Y., and Rosenberg, E. (1999) Inhibition of photosynthesis and bleaching of zooxanthellae by the coral bleaching bacterium *Vibrio shiloi*. *Environ Microbiol* **1:** 223–229.

Colwell, R.R. (1996) Global climate and infectious disease: the cholera paradigm. *Science* **274:** 2025–2031.

Fine, M., Oren, U., and Loya, Y. (2002) Bleaching effect on regeneration and resource translocation in the coral *Oculina patagonica*. *Mar Ecol Progr Series* **234:** 119–125.

Huq, A., Xu, B., Chowdbury, M.A., Islam, M.S., Montilla, R., and Colwell, R.R. (1996) A simple filtration method to remove plankton-associated *Vibrio cholerae*. raw water supplies in developing countries. *Appl Environ Microbiol* **62:** 2508–2512.

Israely, T., Banin, E., and Rosenberg, E. (2001) Growth, differentiation and death of *Vibrio shiloi*. coral tissue as a function of seawater temperature. *Aquat Microbial Ecol* **24:** 1–8.

Kushmaro, A., Loya, Y., Fine, M., and Rosenberg, E. (1996) Bacterial infection and coral bleaching. *Nature* **380:** 396.

Kushmaro, A., Rosenberg, E., Fine, M., and Loya, L. (1997) Bleaching of the coral *Oculina patagonica* by *Vibrio* AK-1. *Mar Ecol Prog Series* **147:** 159–165.

Kushmaro, A., Banin, E., Stackebrandt, E., and Rosenberg, E. (2001) Vibrio shiloi *sp. nov. the causative agent of bleaching of the coral* Oculina patagonica. *IJSEM* **51:** 1383–1388.

Lewis, J.B., and Crooks, R.E. (1996) Foraging cycles of the amphinomid polychaeta *Hermodice caranculata* preying on the calcareous hydrozoan *Millipora complanata*. *Bull Mar Sci* **58:** 853–856.

Manz, W., Arp, G., Schumann-Kindel, G., Szewzyk, U., and Reiner, J. (2000) Widefield deconvolution epifluorescence microscopy combined with fluorescence *in situ* hybridization reveals the spatial arrangement of bacteria in sponge tissue. *J Microbial Meth* **40:** 125–134.

Pernthaler, J., Glöckner, O., Unterholzner, S., Alfreider, A., Psenner, R., and Amann, R. (1998) Seasonal community and population dynamics of pelagic bacteria and archaea in a high mountain lake. *Appl Environ Microbiol* **64:** 4299–4306.

Rattenbury-Marsden, J. (1963) The digestive tract of *Hermodice caranculata* (Pallas). Polychaeta: Amphinomidae. *Can J Zool* **41:** 165–184.

Rosenberg, E., and Ben-Haim, Y. (2002) Microbial diseases of corals and global warming. *Environ Microbiol* **4:** 318–326.

Rozhak, D.B., and Colwell, R.R. (1987) Survival strategies of bacteria in the natural environment. *Microbiol Rev* **51:** 365–379.

Witman, J.D. (1998) Effects of predation by the fireworm *Hermodice caranculata* on milleporid hydrocorals. *Bull Mar Sci* **42:** 446–458.