Bacterial infection and coral bleaching

SIR — Bleaching in stony corals is the result of disruption of symbioses between the coral hosts and photosynthetic microalgal endosymbionts (zooxanthellae). Coral bleaching of unprecedented frequency and global extent were reported in the 1980s and early 1990s[1–3]. This process may be evoked by various environmental stimuli, including increased seawater temperature[4] and ultraviolet radiation[5]. There has been speculation that large-scale bleaching episodes are linked to global warming[6,7]. The data presented here demonstrate for the first time that coral bleaching, in this case, bleaching of *Oculina patagonica*, is caused by a bacterial infection.

We first observed bleaching of *O. patagonica* in the summer of 1993. Bleached patches of polyps appeared throughout the affected colonies (*a* in the figure). Although the tentacular rim of the polyp may retain its pigmentation, the extratentacular coenosarc of the tissue was bleached, showing a total loss of pigmentation (*b* in the figure). Histological sections of bleached tissues showed a 70–90% reduction of algal densities. The initial observation that led us to consider that coral bleaching may be due to bacterial infection was the presence of large aggregates of rod-shaped bacteria on the border of the bleached zone at the tentacular rim (*c* in the figure). In unbleached tissues, no bacterial aggregates were observed.

We collected bleached and unbleached corals from the Mediterranean coast of Israel and 'streaked' samples onto marine agar. Characteristic cream-coloured bacterial colonies, which we call strain AK-1, appeared on all eight samples from bleached corals and were absent from all 14 unbleached corals. Strain AK-1 has been classified as a *Vibrio* by standard microbiological tests[8].

Inoculation of healthy *O. patagonica* with pure cultures of *Vibrio* AK-1 resulted in bleaching. We performed two types of experiment. First, 5×10^6 bacteria were placed on each of five healthy corals, and the corals then put back in an aquarium maintained at 25 °C. All the corals showed bleaching after 6–8 days. In a control experiment in which sterile medium was used in place of the bacteria, none of the corals showed any signs of bleaching. Bleaching was due to the bacterial cells themselves, and not extracellular products, because ultrafiltration led to an inactive cell-free supernatant fluid. The washed cells were as active as the original culture.

The remaining experiments were carried out at 26 °C without removing the corals from two-litre aerated aquaria. In the first test, we added 10^5 *Vibrio* AK-1 per ml to two aquaria containing *O. patagonica*. Antibiotics were added to one of these infected aquaria. A third control aquarium was treated in exactly the same manner, except that we added sterile medium in place of bacteria. After 44 days, 90% of the coral surface in the experimental aquarium was bleached, whereas no bleaching was observed in the control and the antibiotic-treated aquarium (*d* in the figure). In a second test, three aquaria were infected with 5×10^6 *Vibrio* AK-1 per ml and two additional aquaria served as controls. In all three experimental aquaria, there was extensive bleaching after 42 days and tissue retraction after 52 days. The two control colonies remained healthy. Attempts to infect corals with 10^5 *Vibrio* AK-1 per ml at 16 °C were unsuccessful.

Our findings demonstrate that the causative agent of bleaching of *O. patagonica* is *Vibrio* AK-1, which was present in all bleached *O. patagonica* examined, obtained in pure culture and caused bleaching when inoculated onto healthy (unbleached) corals. Seawater temperature is a contributing factor; an increase in temperature may influence the outcome of the infection by lowering the resistance of the coral and increasing the virulence of the bacterium.

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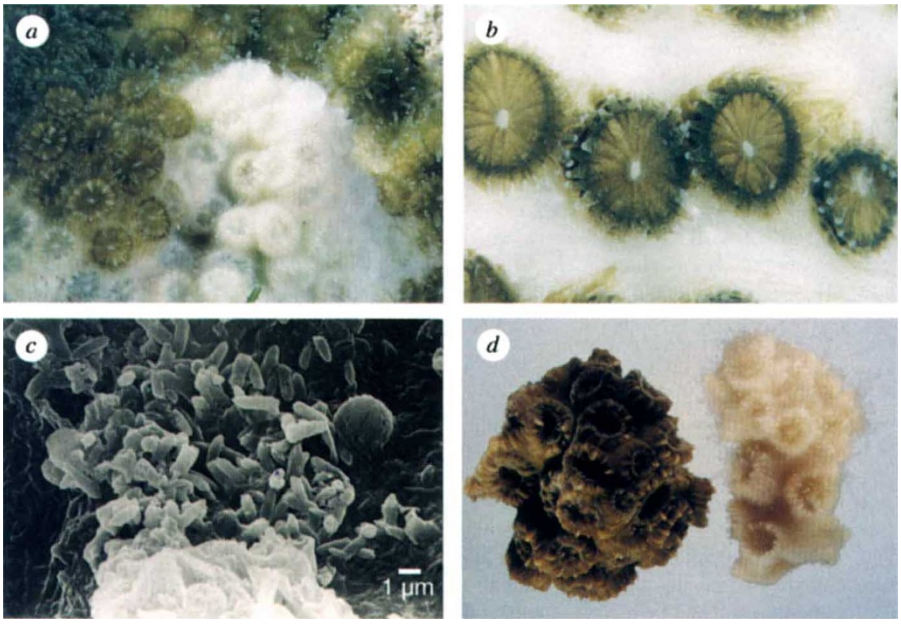
1. Williams, E., Goenaga, C. & Vicente, V. *Science* **238,** 877–878 (1987).
2. Brown, B. E. (ed.) in *Coral Reefs* Spec. Iss. **8,** 153–232 (1990).
3. Glynn, P. W. *Trends Ecol. Evol.* **6,** 175–179 (1991).
4. Jokiel, P. L. & Coles, S. L. *Coral Reefs* **8,** 155–162 (1990).
5. Gleason, D. F. & Wellington, G. M. *Nature* **365,** 836–838 (1993).
6. Smith, S. V. & Buddemeier, R. W. *A. Rev. Syst.* **23,** 89–118 (1992).
7. Glynn, P. W. *Coral Reefs* **12,** 1–17 (1993).
8. Farmer, J. J. III & Hickman-Brenner, F. W. in *The Prokaryotes* 2nd edn (eds Balows, A. *et al.*) 2952–3011 (Springer, New York, 1992).



The coral *Oculina patagonica*. *a*, Bleached patches of polyps throughout the affected colony (×2). *b*, Extratentacular coenosarc of the bleached tissue showing a total loss of pigmentation (×5). *c*, Scanning electron microscopy showing large aggregates of rod-shaped bacteria on the border between the bleached and unbleached zones at the tentacular rim. *d*, Control: healthy coral (left) and a bleached coral (right) 44 days after infection with bacterial strain AK-1 (×2). Photographs by A. Shoob.

Pest control by fluorescence

SIR — Environmentally safe biological control agents, such as baculoviruses, are among the most promising alternatives to synthetic chemical insecticides. Among the deterrents to their commercial development has been the lack of cost-effective procedures for monitoring their efficacy and ecology in nature. Green fluorescent protein (GFP) is a very stable protein which emits green light on excitation with ultraviolet or blue light at 395 or 470 nm (ref. 1). We have transferred the GFP gene from the jellyfish *Aequorea victoria* into a typical baculovirus, the *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV)[2], thus producing an easily visible marker for detecting infected insects.

We excised a complete GFP-coding sequence from plasmid pGFP (Clontech) and inserted it into the polyhedrin gene-containing transfer vector pAcUW21