Coral Microbiology

BY EUGENE ROSENBERG, CHRISTINA A. KELLOGG, AND FOREST ROHWER

In the last 30 years, there has been approximately a 30% loss of corals worldwide, largely due to emerging diseases (Harvell et al., 2002, 2007; Hughes et al., 2003). Coral microbiology is a new field, driven largely by a desire to understand the interactions between corals and their symbiotic microorganisms and to use this knowledge to eventually prevent the spread of coral diseases.

The last decade has brought substantial progress in determining the abundance and diversity of microorganisms associated with healthy and diseased corals. Much of this progress is the result of the introduction of culture-independent molecular techniques into coral microbiology (Rohwer et al., 2001). Consideration of the data published to date leads to the hypothesis that environmental stress factors contributing to coral disease, such as climate change, water pollution, and overfishing, exert

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their effects by altering the microbial population and causing certain microbes to become pathogens.

MICROORGANISMS ASSOCIATED WITH HEALTHY CORALS

The coral holobiont (host organism plus all of its associated microorganisms) is a complex system containing microbial representatives of all three domains: Eucarya, Bacteria, and Archaea, as well as numerous viruses (Figure 1). We discuss the microorganisms associated with healthy coral communities in the next few sections, and then turn to the coral diseases related to microbes.

Eucarya (Zooxanthellae)

Corals contain endosymbiotic dino-flagellates (algae) of the genus *Symbiodinium*, commonly referred to as zooxanthellae. Zooxanthellae provide a large part of their hosts' energy requirements by transferring photo-synthetically fixed carbon to the coral (Fallowski et al., 1984). The algae also produce large amounts of molecular oxygen, which allow for efficient respiration by the coral and associated pro-karyotes. We now know from molecular evidence that the genus *Symbiodinium* is diverse, containing more than seven principal clades, each of which con-
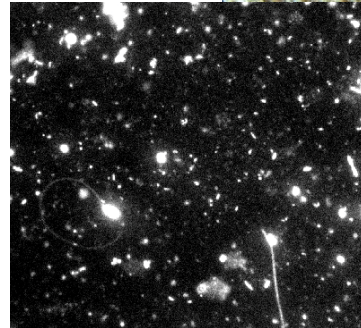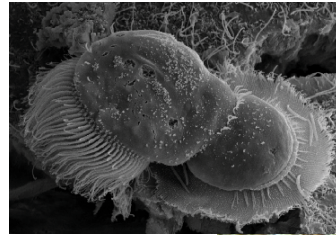
tains multiple "taxa" (Baker, 2003). A single species of coral can host multiple types of *Symbiodinium*, often containing different clades at different depths (Rowan and Knowlton, 1995). Reviews of *Symbiodinium* diversity, systematics, and relationships to coral diseases are presented by Baker (2003, 2004).
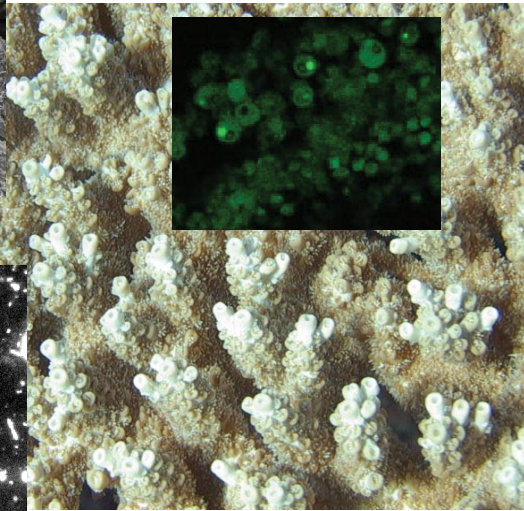
Bacteria

Corals provide three general habitats for bacteria: the surface mucus layer, coral tissue, and the calcium carbonate skeleton, each of which has a distinct bacterial population (Bourne and Munn, 2005; Koren and Rosenberg, 2006). It is likely that within each of these habitats there are microniches. Initially, research in coral microbiology concentrated on the mucus fraction, using culturing techniques. These studies demonstrated that the mucus harbors a diverse and abundant bacterial community (Ducklow and Mitchell, 1979; Ritchie and Smith, 1997), including nitrogen fixers (Shashar et al., 1994; Lesser et al., 2004). The concentration of colony-forming bacteria in the mucus is 10^5–10^6 per ml (Koren and Rosenberg, 2006; Ducklow and Mitchell, 1979), about 0.2% of the total counts determined microscopically using a common technique for detecting micro-organisms called SYBR Gold Staining

Protist

Zooxanthellae

Endolithic Algae

Viruses, Bacteria, & Archaea

Figure 1. The coral holobiont consists of the coral animal and associated viruses, microbes (both bacteria and archaea), zooxanthellae, and endolithic organisms. Most of the viruses are phages, which attack the microbes. The endolithic organisms include algae, fungi, sponges, and microbes that bore into the coral skeleton. The holobiont likely occupies different coral reef niches by mixing and matching different components (i.e., the Probiotic Hypothesis). *Zooxanthellae and protist micrographs by Linda Wegley and Ian Hewson, respectively*

(Koren and Rosenberg, 2006).

Bacteria can also be found in coral tissue after removal of the mucus and skeleton. The numbers of culturable and total tissue bacteria are similar to those found in mucus. However, the abundant bacterial species associated with coral tissue is very different from those found in mucus (Bourne and Munn, 2005; Koren and Rosenberg, 2006).

Coral skeletons are porous structures inhabited by a variety of bacteria. This endolithic community may satisfy 50% of the nitrogen required by the coral (Ferrer and Szmant, 1988). Cyanobacteria in the skeleton of *Oculina patagonica* provide photoassimilates to the coral tissue (Fine and Loya, 2002). These may be critical for the survival of the coral during times when it loses its endosymbiotic algae, a disease referred to as coral bleaching.

Culture-free, DNA-based techniques first applied by Rohwer et al. (2001) to the study of coral bacteria provide useful data on the bacterial community associated with different corals (Bourne and Munn, 2005; Koren and Rosenberg, 2006; Pantos et al., 2003; Rohwer et al., 2001, 2002; Frias-Lopez et al., 2002; Cooney et al., 2002; Sekar et al., 2006; Casas et al., 2004). Certain generalizations are beginning to emerge: (1) the diversity of bacterial species associated with a particular coral species is high, including a majority of novel species; (2) bacteria in a particular coral community are different from those in the seawater surrounding the coral, suggesting the association is specific; (3) the species composition of the uncultured bacterial population is completely different from the cultured population; (4) similar bacterial populations are found on the same coral species that are geographically separated, while different populations are found on different coral species; and (5) different populations are found in mucus and tissue from the same coral fragment.

Archaea

Previously pigeonholed as extremophiles, archaea have been found in a number of mesophilic marine niches: coastal waters (DeLong 1992; Fuhrman et al., 1992), marsh sediments (Munson et al., 1997), fishes (van der Maarel et al., 1998, 1999), sponges (Preston et al., 1996; Margot et al., 2002; Webster et al., 2001), a sea cucumber (McInerney et al., 1995), and several species of corals (Kellogg, 2004; Wegley et al., 2004). Like coral-associated bacterial communities, these archaeal communities are distinct from those in surrounding seawater,

they consist of undescribed species, and different populations appear to inhabit the mucus and the tissue. The mucus samples were dominated by euryarchaeotes (Kellogg, 2004) and the tissue samples by crenarchaeotes (Wegley et al., 2004). Unlike zooxanthellae and bacteria, however, there is no evidence of species-specific associations between particular archaea and individual corals. In fact, there appear to be archaeal generalists; two independent studies found major archaeal ribotypes present in multiple species of coral (Kellogg, 2004; Wegley et al., 2004). Although both studies included the coral *Diploria strigosa*, they had no archaea in common. This may be due to a population difference between mucus and tissue or it may indicate a biogeographical distinction between the study sites (U.S. Virgin Islands versus Panama and Bermuda).

Viruses

Viruses are common components of the coral reef environment. Seymour et al. (2005) used flow cytometry to show that there are ~ 5×10^5 virus-like particles (VLP) in the water above corals. Microscale profiles show that VLP abundance is significantly higher closest to the coral surface. It also appears that VLP concentrations are highest above healthy and diseased corals, in comparison to coral rubble (Patten et al., 2006). The authors suggest that there are fundamental shifts in the VLP and bacterial community in the water associated with diseased corals. Most of the coral reef-associated VLPs are phages—viruses that infect bacteria (recent work of author Rohwer and Rebecca Thurber, San Diego State University). However, the effects of phages on the food-web structure of the coral reef and coral holobiont still need to be determined.

Wilson et al. (2001) show that there is a transferable infectious agent, probably a virus, that can cause bleaching in a sea anemone. In a follow-up study, the corals *Pavona danai*, *Acropora formosa,* and *Stylophora pistillata* were shown to produce numerous VLPs when heat shocked (Wilson et al., 2005). VLPs were also observed in heat-shocked zooxanthellae. Together, these results suggest a mechanism by which viruses might be involved in coral bleaching. However, the field of coral virology is still extremely understudied and a major effort to uncover the diversity and identity of coral viruses present in healthy versus stressed corals is desperately needed (Davy et al., 2006).

INFECTIOUS DISEASES OF CORALS

Of more than twenty coral diseases described by coral biologists, the causative agent for only six of the diseases has been isolated and characterized (Table 1, Figure 2).

Coral Bleaching

At the global scale, coral bleaching is the most serious disease threatening coral reefs. Coral bleaching is the disruption of symbioses between coral hosts and their endosymbiotic *Symbiodinium*. The loss of the microalgae and/or its photosynthetic pigments causes the coral to lose color in this bleaching process. If the process is not reversed within a few weeks or months, depending on the specific coral species and the environmental conditions, the coral will die. In general,

Table 1. Coral Pathogens

Disease	Pathogen	Coral Host	Reference
Bleaching	*Vibrio shiloi*	*Oculina patagonica*	Kushmaro et al., 1996, 1997
Bleaching and lysis	*Vibrio coralliilyticus*	*Pocillopora damicornis*	Ben-Haim and Rosenberg, 2002; Ben-Haim et al., 2003
Aspergillosis	*Aspergillus sidowii*	Gorgonians (sea fans)	Smith et al., 1996, Geiser et al., 1998
White band	*Vibrio carchariae**	*Acropora* sp.	Ritchie and Smith, 1995
White plague	*Aurantimonas coralicida*	Several	Denner et al., 2003
White plague (Eilat)	*Thalassomonas loyana*	Several	Barash et al., 2005; Thompson et al., 2006; Efrony et al., 2007
White pox	*Serratia marcenscens*	*Acropora palmata*	Patterson et al., 2002
Yellow blotch	*Vibrio alginolyticus*	*Monastraea* sp.	Cervinio et. al., 2004
Black band	Consortium* (?)	Several	Richardson, 2004

* Presumed pathogens, but Koch's postulates not demonstrated

coral bleaching coincides with the hottest period of the year (Goreau et al., 1997; Hayes and Goreau, 1998) and is most severe at times of warmer-than-normal conditions (Hoegh-Guldberg, 1999).

Shortly after bleaching of *O. patagonica* in the Mediterranean was first reported in 1995, the causative agent of the disease was identified as *Vibrio shiloi* (Kushmaro et al., 1996; Kushmaro et al., 1997). Increased seawater temperature was the environmental factor that triggered the disease (Kushmaro et al., 1998). Infection and resulting bleaching only occurred at summer seawater temperatures (25–30°C) and not at winter temperatures (16–20°C). The mode of infection and the temperature dependence of many steps in the process have been studied extensively (Rosenberg and Falkowitz, 2004). The winter reservoir and summer vector for transmitting the disease is the marine fireworm *Hermodice carunculata*

(Sussman et al., 2003).

Vibrio coralliilyticus is an etiological agent of bleaching of the coral *Pocillopora damicornis* on coral reefs in the Indian Ocean and Red Sea (Ben-Haim and Rosenberg, 2002; Ben-Haim et al., 2003). The infection of *P. damicornis* by *V. coralliilyticus* shows strong temperature dependence. Below 22°C, no infection occurs. At 24–26°C, the infection results in bleaching, whereas at 27–29°C the infection causes rapid tissue lysis and death of the coral.

White Band Disease

White band disease is characterized by a progressive lesion starting from the base of the coral and moving to the tip (Figure 2c). Ritchie and Smith (1995) compared the microbial community structure of diseased and healthy corals using carbon source utilization pattern analyses. They found that diseased corals contained a much higher propor-

tion of vibrios. These disease-associated vibrios clustered most closely to *Vibrio carchariae*. Because infection experiments with isolated *V. carchariae* were not reported, it is not clear whether or not it is the causative agent or the result of the disease. Culture-independent analyses of white band disease type I and healthy Acropora species showed that the microbial community was dominated by a single bacterial species related to Rickettsiales. However, there were no dramatic differences in the bacterial communities associated with white band disease. These results suggest that a nonbacterial pathogen might be the cause of the disease (Casas et al., 2004).

White Plague Disease

In the summer of 1995, a devastating disease occurred on reefs of the northern Florida Keys, spreading rapidly to infect 17 different coral species. The disease, designated white plague, was charac-

a. *Oculina patagonica*   b. *Gorgonia ventalina*   c. *Acropora cervicornis*   d. *Diploria strigosa*

e. *Favia favius*   f. *Acropora palmata*   g. *Montastraea faveolata*   h. *Diploria strigosa*
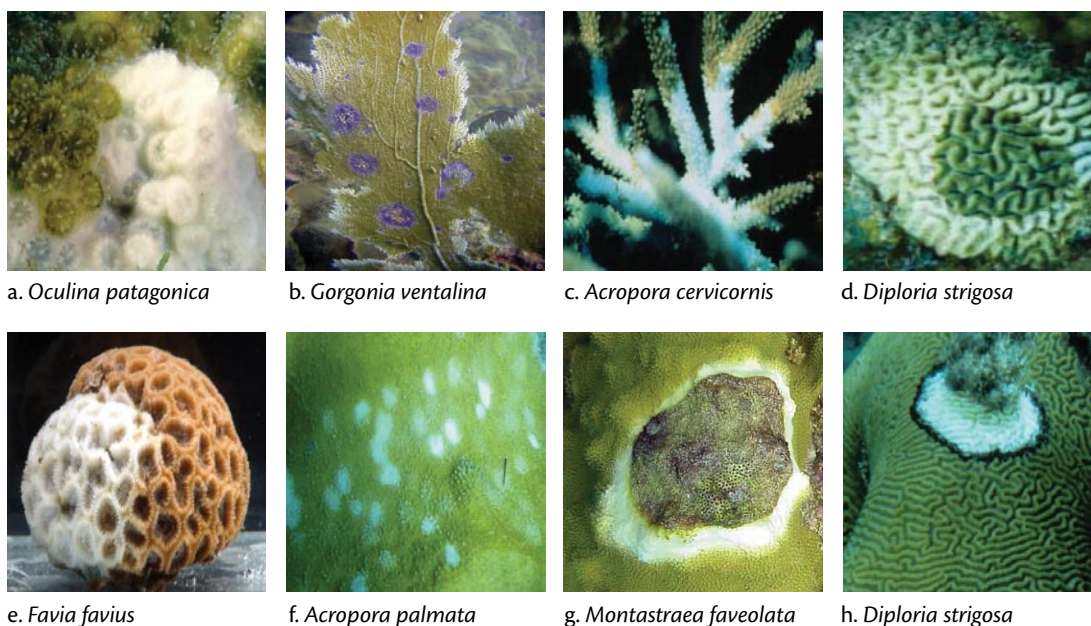
Figure 2. Photographs of diseased corals. (a) Bleaching of *Oculina patagonica*. (b) Aspergillosis of *Gorgonia ventalina*. (c) White band disease of *Acropora cervicornis*. (d) White plague disease of *Diploria strigosa*. (e) White plague disease of *Favia favius*. (f) White pox disease of *Acropora palmata*. (g) Yellow blotch disease of *Monastraea faveolata*. (h) Black band disease of *Diploria strigosa*. L. Richardson and NOAA provided photographs b, c, d, f, g, and h

terized by a sharp line between freshly exposed coral skeleton and apparently healthy tissue (Figure 2d). The lesion progressed rapidly (up to 2 cm/day), leading to mortality within 10 weeks. Microbiological studies indicated that

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the causative agent was a single Gram-negative bacterium. Subsequently, this pathogen was classified as a representative of a hitherto unknown marine taxon of the order Rhizobiales of the class alpha-Proteobacteria and called *Aurantimonas coralicida* (Denner et al., 2003).

Starting in 2003, a white plague-like disease (Figure 2e) spread over the Eilat coral reef, Gulf of Aqaba, Red Sea, affecting approximately 10% of the major reef-building coral genera. The pathogen was identified as a new species, *Thalassomonas loyana* (Barash et al., 2005; Thompson et al., 2006). To demonstrate unequivocally that *T. loyana* is the causative agent of the white plague-like disease on the Eilat coral reef, an indirect, but powerful, novel method was employed—phage therapy (Efrony et al., 2007). A bacteriophage specific for *T. loyana* was isolated and shown to block the disease.

White Pox Disease
Serratia marcescens causes white pox disease (Figure 2f) in the common Caribbean reef-building coral, *Acropora palmata* (Patterson et al., 2002). *S. marcescens*, a Gram-negative coli-form bacterium and a member of the Enterobacteriacae family, is a known pathogen of humans, cows, goats, chickens, fish, insects, and plants. This is the first example of a coral pathogen with a possible link to human sewage pollution.

Yellow Blotch Disease
During the early stages of yellow blotch disease, pale yellow blotches appear on the coral tissue. The blotches expand and form bands (Figure 2g) that can spread slowly across the colony at rates of 0.5–1.0 cm/month (Santavy et al., 1999). After a series of infection/reisolation experiments, four *Vibrio* strains were obtained that induced the disease in controlled aquarium experiments (Cervino et al., 2004). One of the strains was identified as *Vibrio alginolyticus*. The rate of spread of the disease was proportional to temperature from 25–33°C. The *Vibrio* infection targets the endosymbiotic algae rather than the coral tissue, sug-gesting a close similarity to the above-described coral bleaching by *V. shiloi* and *V. coralliilyticus*.

Black Band Disease
Black band disease can be recognized as a dark band that moves across coral colonies, destroying coral tissue (Figure 2h). The disease is most active during the warm summer months (Kuta and Richardson, 2002). Although this disease has been studied extensively, the causative agent remains elusive (Cooney et al., 2002; Frais-Lopez et al., 2002). Microscopic observation of diseased tissue reveals the presence of heterotrophic and photosynthetic bacteria. Richardson (2004) argues for the concept that black band disease is not caused by a single primary pathogen, but rather results from a pathogenic consortium behaving as a microbial mat.

Fungal Infections
During the 1980s, certain areas of the Caribbean experienced mass mortalities of sea fans (Garzon-Ferreira and Zea, 1992), but microbiological studies were not performed. In 1995, sea fans showing disease signs (Figure 2b) similar to those reported in the 1980s were observed throughout the Caribbean (Nagelkerken et al., 1997). The causative agent of this disease was isolated and identified by classical and molecular methods as a strain of the fungus *Aspergillis sydowii* (Smith et al., 1996; Geiser et al., 1998). Aspergilli are considered terrestrial fungi and do not sporulate in seawater. Two hypotheses have been put forth to explain the source of the coral pathogen (Smith and Weil, 2004): runoff from the land and African dust events.

The presence of endolithic fungi in scleractinian corals has been known since the 1800s (Kölliker, 1859). These fungal associates have been described from many geographic areas and species, and seem to infect the coral at an early stage of development (Bentis et al., 2000). Although healthy corals appear to be able to keep endolithic fungal attacks on polyps in check by walling them off (Le Campion-Alsumard et al., 1995), stresses such as increased water temperature, bleaching, or disease provide an opportunity for secondary infection, for example, fungal infection associated with black band disease (Ramos-Flores, 1983) and pink line syndrome (Ravindran et al., 2001). Most of these fungi remain partially or completely unidentified because characterization is based on histological and microscopic examination of the hyphae that is hampered by a lack of sporulation in cultured isolates. Molecular techniques have not yet been successfully applied to this task because nominally fungal-specific primer sets also amplify the 18S rDNA gene from the coral host.

Archaeal Pathogens?

As studies increasingly reveal the diversity of archaea and the varied environments they inhabit, it is interesting that no archaea have been characterized as pathogens. Archaea have been shown to colonize metazoans, including all ruminants, humans (Kulik et al., 2001; Miller and Wolin, 1982), fish (van der Maarel et al., 1998, 1999), and corals (Kellogg, 2004; Wegley et al., 2004). It has been suggested that horizontal transfer of genes from archaea to bacterial pathogens may have enhanced bacterial viru-

lence (Gophna et al., 2004). A link has been made between the relative abundance of methanogenic archaea in the subgingival crevice and the severity of periodontal disease in humans (Lepp et al., 2004). The methogens do not cause the disease, but their metabolism produces a conducive environment for certain bacteria. Considering how little we know about archaeal metabolic capabilities, and that corals have $> 10^7$ archaea per cm^2 in addition to at least as many bacteria (Wegley et al., 2004), corals would seem to be an excellent laboratory to begin studying these interactions and the possible pathogenicity of archaea.

STRESS FACTORS THAT CONTRIBUTE TO CORAL DISEASE

Climate change (Harvell et al., 2002, 2007), water pollution (Szmant, 2002), and overfishing (Jackson et al., 2001) are the three most frequently cited environmental stress factors responsible for the rise in coral disease. Until recently, evidence supporting these claims has been largely circumstantial, namely, correlations between these stress factors and the frequency of disease. However, recent studies provide direct experimental evidence demonstrating how each of these factors contributes to coral disease.

Climate Change

Earth is undergoing accelerating climate change that is being driven by increasing concentrations of greenhouse gases. During the last century, the average global temperature increased $0.6 \pm 0.2°C$, and it is predicted to rise another $1.5–4.5°C$ this century (IPCC, 2007). All of the coral diseases shown in Figure 2 occur more frequently and progress

more rapidly in warm summer months. In the two documented examples of bacterial bleaching of corals, the mechanisms by which temperatures affect the process were established. As noted above, expression of critical bacterial virulence genes is temperature dependent.

Water Pollution

Elevated nutrients (e.g., phosphate, nitrate, ammonia, and dissolved organic carbon) in coastal waters have been suggested as a cause of reef decline. Field experiments using a slow-release fertilizer in nylon bags demonstrated that increasing inorganic nitrogen and phosphate by two to five times increased the severity of aspergillosis and yellow blotch diseases (Bruno et al., 2003). Addition of small quantities of carbohydrates to corals in a flow-though system caused substantial coral mortality and an order of magnitude increase in the growth rate of microorganisms in the coral mucus (Kline et al., 2006). The latter study suggests that mortality results from a disruption of the balance between the coral and its associated microbiota. These studies should be valuable in implementing coral monitoring and management plans.

Overfishing

Overfishing reduces the number of fish that graze on algae, thus increasing the concentration of algae present on coral reefs. Numerous studies show that various species of algae can influence corals negatively (reviewed by McCook et al., 2001). Suggested mechanisms include alleopathy, smothering, shading, abrasion, overgrowth, and harboring potential pathogens. Smith et al. (2006)

demonstrate that algae can cause coral mortality by enhancing microbial activity via the release of dissolved compounds. Coral mortality can be completely prevented by addition of antibiotics, thus supporting the hypothesis that the microbial community is the agent of coral death under conditions of abundant algal growth.

CORAL RESISTANCE TO STRESS AND THE CORAL PROBIOTIC HYPOTHESIS

Although corals contain innate or non-specific immunity systems, they do not produce antibodies and are considered to lack an adaptive immune system. However, recent data demonstrate that corals can develop resistance to specific pathogens and adapt to higher temperatures. The Coral Probiotic Hypothesis (Reshef et al., 2006), discussed below, explains these findings within the context of what is currently known about coral microbiology.

Adaptation of Corals to Stress

From 1995 to 2002, bacterial bleaching of the coral *O. patagonica* in the eastern Mediterranean occurred every summer. During that time period, *V. shiloi* was repeatedly isolated from bleached corals and shown to cause bleaching of healthy corals in controlled aquarium experiments. However, since 2003, it has not been possible to isolate *V. shiloi* from bleached or healthy corals, and all laboratory stocks of *V. shiloi* failed to cause bleaching of fresh, healthy corals taken from the sea (Reshef et al., 2006). There are other, less-well-documented examples of coral communities that have become resistant to specific pathogens.

For example, *A. coralicida* (Denner et al., 2003), the bacterium responsible for the white plague disease on reefs off the Florida Keys in 1995, can no longer infect these corals (Richardson and Aronson, 2002).

The Coral Holobiont and Probiotic Hypothesis

The ability to adapt to stresses, such as high temperature and infection by specific pathogens, has led to the Coral Probiotic Hypothesis (Reshef et al., 2006). This hypothesis posits that a dynamic relationship exists between symbiotic microorganisms and corals at different environmental conditions, which bring about a selection for the most advantageous coral holobiont. The hypothesis is similar to the Adaptive Bleaching Hypothesis for zooxanthellae but more inclusive because the coral holobiont is defined as the host organism plus *all* of the associated microorganisms. By changing its microbial community, the holobiont can adapt to changing environmental conditions more rapidly (weeks to years) than it can via mutation and selection of the coral host itself (thousands of years). Four key observations discussed in this article support the Coral Probiotic Hypothesis: (1) corals contain a large and diverse microbial population associated with their mucus and tissues; (2) coral-associated microorganisms can benefit their host by a variety of different mechanisms (e.g., nitrogen fixation, photosynthesis, and production of antimicrobials); (3) the coral-associated microbial population undergoes a rapid change when environmental conditions are altered; and (4) although

lacking an adaptive immune system (no antibodies), corals can develop resistance to pathogens. The Coral Probiotic Hypothesis may help explain the evolutionary success of corals and moderate predictions of their demise.

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