The Role of Vibrios in Diseases of Corals

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ABSTRACT The tissue, skeleton, and secreted mucus of corals supports a highly dynamic and diverse community of microbes, which play a major role in the health status of corals such as the provision of essential nutrients or the metabolism of waste products. However, members of the Vibrio genus are prominent as causative agents of disease in corals. The aim of this chapter is to review our understanding of the spectrum of disease effects displayed by coral-associated vibrios, with a particular emphasis on the few species where detailed studies of pathogenicity have been conducted. The role of Vibrio shilonii in seasonal bleaching of Oculina patagonica and the development of the coral probiotic hypothesis is reviewed, pointing to unanswered questions about this phenomenon. Detailed consideration is given to studies of V. coralliilyticus and related pathogens and changes in the dominance of vibrios associated with coral bleaching. Other Vibrio-associated disease syndromes discussed include yellow band/blotch disease and tissue necrosis in temperate gorgonian corals. The review includes analysis of the role of enzymes, resistance to oxidative stress, and quorum sensing in virulence of coral-associated vibrios. The review concludes that we should probably regard most—possibly all—vibrios as "opportunistic" pathogens which, under certain environmental conditions, are capable of overwhelming the defense mechanisms of appropriate hosts, leading to rapid growth and tissue destruction.

INTRODUCTION

Corals are members of the Anthozoa clade of the phylum Cnidaria. Individual coral animals take the form of roughly cylindrical polyps, comprising a sac surrounding a gastrointestinal cavity, with tentacles for the capture of prey. The best known corals are the stony corals (Scleractinia) that usually associate into colonies, many species of which contain symbiotic photosynthetic dinoflagellate Symbiodinium algae (zooxanthellae) that fix CO₂ into organic compounds utilized by the coral host for growth. In many coral species, the excess production of fixed carbon is critical to the formation of an external skeleton of calcium carbonate, leading to the formation of reefs, and the secretion of carbon-rich mucus, which provides a major source of nutrients to microbial processes in the reef ecosystem (1). These properties mean that corals play a major role in the maintenance of marine biodiversity and have great economic importance for communities that depend on them for coastal protection, fishing, tourism, and other activities. Coral reefs are under severe threat from global climate change, ocean acidification, pollution, overfishing, and disease, and many coral biologists predict that they will be irreversibly damaged during this century (2).

During the last decade, we have realized that the tissue, skeleton, and secreted mucus of corals supports a highly dynamic and diverse community of microbes, leading to the concept of coral as a holobiont (3, 4, 5), as illustrated in Fig. 1. Besides the zooxanthellae, other protists, Bacteria, Archaea, Fungi, and viruses all play a major role in the health status of corals by provision of essential nutrients, by metabolism of waste or toxic products, or by inhibition of pathogens (6, 7, 8). However, some organisms are pathogenic and coral diseases have been described in all the major reef systems of the world, involving numerous coral species (9), as well as in
aquarium corals (10). Many coral diseases are poorly documented and details of pathology and specific causation are often lacking. However, of the 18 or so well-recognized syndromes in which disease lesions have been well-described (3), about half have been strongly associated with fungal or bacterial infection. Among these, as shown in Table 1, members of the genus *Vibrio* are particularly prominent as possible causative agents. In addition, a wide range of other vibrios in the genera *Vibrio*, *Photobacterium*, and *Enterovibrio* have been cultured from the tissue and mucus of “healthy” corals (i.e., those with no obvious disease signs) using traditional methods, such as isolation on marine agar and the selective thiosulfate-citrate-bile-salts-sucrose (TCBS) agar (e.g., 11, 12, 13, 14, 15, 16, 17, 18, 19, 20). Use of nonculture-based methods, such as denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (TRFLP) analysis, clone library analysis, or metagenomics, has also led to recognition of sequences homologous to those of known vibrios in a range of studies of coral microbial communities in the absence of overt disease (e.g., 21, 22, 23, 24, 25). Many of the *Vibrio* spp. noted in Table 1 possess enzymes, toxins and other virulence factors, but there is

![FIGURE 1](https://doi.org/10.1128/microbiolspec.VE-0006-2014.f1)

**TABLE 1** Principal disease of corals for which there is a strong association with fungal or bacterial infection

<table>
<thead>
<tr>
<th>Disease/syndrome</th>
<th>Associated pathogen(s)</th>
<th>Principal host(s)</th>
<th>Location</th>
<th>Key references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillosis</td>
<td><em>Aspergillus sydowii</em></td>
<td>Gorgonia spp. and other octocorals</td>
<td>C</td>
<td>(101)</td>
</tr>
<tr>
<td>Black band disease</td>
<td><em>Phormidium corallyticum</em>, <em>Desulfovibrio</em> spp. and <em>Beggiatoa</em> spp.</td>
<td>Wide range</td>
<td>C IP</td>
<td>(102, 103, 104)</td>
</tr>
<tr>
<td>Bleaching</td>
<td><em>Vibrio shilonii</em> (originally classified as V. shiloii)</td>
<td><em>Oculina patagonica</em></td>
<td>M</td>
<td>(35)</td>
</tr>
<tr>
<td>Bleaching and tissue lysis (white syndrome in IP)</td>
<td><em>Vibrio coralliilyticus</em>, <em>Vibrio harveyi</em>, <em>Vibrio owensii</em></td>
<td><em>Pocillopora damicornis</em>, <em>Montipora</em> spp., <em>Acropora</em> spp., <em>Pachyseris</em> speciosa</td>
<td>C IP RS</td>
<td>(57, 58, 105, 106)</td>
</tr>
<tr>
<td>Rapid tissue necrosis (shut down reaction)</td>
<td><em>Vibrio alginolyticus</em>, <em>Vibrio harveyi</em></td>
<td>Wide range</td>
<td>A C IP RS</td>
<td>(107)</td>
</tr>
<tr>
<td>White band</td>
<td><em>Vibrio caranchiae</em>, <em>Vibrio harveyi</em></td>
<td><em>Acropora</em> spp.</td>
<td>C</td>
<td>(108)</td>
</tr>
<tr>
<td>White plague</td>
<td><em>Thalassomonas loyana</em></td>
<td><em>Favia</em> favus</td>
<td>RS</td>
<td>(109)</td>
</tr>
<tr>
<td>White plague type II</td>
<td><em>Aurantimonas coralica</em></td>
<td><em>Dichocenia</em> spp. and others</td>
<td>C</td>
<td>(110)</td>
</tr>
<tr>
<td>White pox</td>
<td><em>Serratia marcescens</em></td>
<td><em>Acropora</em> palmata</td>
<td>C</td>
<td>(111)</td>
</tr>
<tr>
<td>Yellow band/blotch</td>
<td><em>Vibrio rotiferanus</em>, <em>Vibrio harveyi</em>, <em>Vibrio alginolyticus</em>, <em>Vibrio proteolyticus</em></td>
<td><em>Acropora</em> spp., <em>Montastrea</em> spp., <em>Diplastrea</em> spp., <em>Fungia</em> spp.</td>
<td>C</td>
<td>(83)</td>
</tr>
</tbody>
</table>

1 A, aquaria; C, Caribbean Sea; IP, Indo-Pacific Ocean; M, Mediterranean Sea; RS, Red Sea.
disagreement about whether they should be regarded as primary causal agents (26). Other vibrios appear to be members of the “normal” microbial community, with no known links to disease (16); some contribute to nitrogen fixation (27, 28) and defense of the coral against pathogens (29). The aim of this chapter is to review our understanding of the spectrum of disease effects displayed by coral-associated vibrios, with a particular emphasis on the few species where detailed studies of pathogenicity have been conducted.

**VIBRIO SHILONII (“V. SHILOI”) AS A CAUSE OF SEASONAL BLEACHING IN A MEDITERRANEAN CORAL**

Coral bleaching is due to physiological disturbances resulting in disruption of the symbiosis between the host and the photosynthetic zooxanthellae (30, 31, 32). The primary trigger is generally considered to be increased temperature (33, 34), although the cellular processes involved are complex and still not fully understood (32). An alternative hypothesis for the initiation of bleaching by bacterial infection emerged from the work of Rosenberg’s group in Tel Aviv, Israel, in the early 1990s. Bleaching of the invasive coral, *Oculina patagonica*, on the Israel coast of the Mediterranean Sea was observed to occur each summer, with a clear correlation to seawater temperature (35). A particular bacterium designated AK1 was isolated from all bleached corals, but not from unbleached corals (36). When *O. patagonica* was inoculated with strain AK1 in aquaria at different temperatures, bleaching occurred rapidly at 29°C; whilst at 20°C or 25°C, the rate of bleaching was slower or incomplete with no bleaching at all (37). Furthermore, addition of the antibiotics penicillin G or kanamycin to the aquarium water inhibited bleaching (37); strain AK1 is susceptible to these antibiotics in vitro (38). This isolate was subsequently identified as a new species named *Vibrio shiloi* (38). The taxonomy of this bacterium has caused some confusion because the spelling of the specific epithet *shiloi* assigned in the original description was corrected by the List Editor to *shilonii* (39). Shortly after the original description, after comparison using a polyphasic approach with phenotypic and genomic characters, Thompson and coworkers (40) argued that there was not sufficient evidence to distinguish “*V. shiloi*” from the previously described *V. mediterranei* and that the proposal for a new species based on virulence was not valid. Despite these points, the names *V. shiloni* (and, more commonly, the incorrect form *V. shiloi*) have continued to be used in many subsequent references. On the basis of multilocus sequence analyses (MLSA), which included the original AK1 strain, Tarazona and colleagues (41) recently concluded that there is “overwhelming evidence of synonymy” of *V. shilonii* and *V. mediterranei*.

Besides the original AK1 strain (deposited as the type strain LMG19703), only one other isolate (AK2, LMG20977) of *V. shilonii* from bleached corals has been deposited in culture collections. Rosenberg’s group subsequently conducted a series of experiments providing compelling evidence of a variety of temperature-regulated factors implicated in the virulence of *V. shilonii* for *O. patagonica*. When *V. shilonii* was grown at 25°C, the bacterial cells adhered rapidly to coral fragments in aquaria, whereas bacteria grown at 16°C did not adhere, regardless of whether the corals were grown at 16°C or 25°C (42). The adhesion was strongly inhibited by D-galactose (or a synthetic analogue of this sugar) suggesting that the coral surface contains a galactoside receptor for the bacterial adhesion (43). It was shown that the proposed receptor was present in the mucus and that the zooxanthellae must be active in forming an ion channel that allows ammonia to disrupt the normal pH gradient. Again, this factor was only produced at the higher temperatures implicated in bleaching (44). The authors suggested that extracellular enzymes are also involved in the bleaching and lysis, but these were not characterized.

The interaction between *V. shilonii* and *O. patagonica* became an elegant model system in Rosenberg’s laboratory. However, since 2004, *V. shilonii* has no longer been detectable in either healthy or bleached corals using culture methods (45), fluorescent in situ hybridization (FISH) (46), or fluorescence microscopy using specific anti-*V. shilonii* antibodies (47). Also, no sequences corresponding to *V. shilonii* were detected in 16S rRNA gene libraries obtained from *O. patagonica* mucus or tissues in 2004 (winter) or 2005 (summer) (48). Subsequent investigations revealed that *O. patagonica* has apparently developed resistance to *V. shilonii* – until 2002, strains of pathogenic *V. shilonii* held in the...
laboratory caused bleaching in aquarium experiments, but the same strains are now incapable of infecting O. patagonica. When corals obtained in 2006 were infected with V. shilonii, the bacteria were observed to adhere to the coral and penetrate into the tissues, but their numbers declined after 24 h and were undetectable within 4 days (47). Because corals lack adaptive immunity, the “coral probiotic hypothesis” (CPH) was proposed to explain this phenomenon (47). The CPH suggests that the relative abundance of members of the microbial community of O. patagonica has changed to produce antagonistic effects that inhibit and kill V. shilonii (47). The concept was later developed as a more general explanation for the evolution of host-symbiont interactions (48). It is obviously impossible to go back in time to investigate what changes may have occurred in O. patagonica, but work in our laboratory has provided some results supporting the concept of a probiotic effect on microbial communities associated with the coral holobiont (48). Nine bacterial strains isolated from O. patagonica mucus in 2007 inhibited the growth of V. shilonii, of which the most potent were a Pseudoalteromonas sp. and a Roseobacter sp. (49). In in vitro experiments, inoculation of liquid cultures or previously established biofilms of the Roseobacter and Pseudoalteromonas strains with V. shilonii led to rapid and significant losses of viability of V. shilonii. Results indicated the production of a diffusible antibiotic compound as well as cell-associated antibacterial activity (49). Besides the CPH, we must consider other possible explanations for the “disappearance” of V. shilonii. It is possible that the properties of the pathogen have changed, rendering it unable to colonize the coral or produce virulence factors. Indeed, genomic analysis of strains and comparison with strains of the closely related V. mediterranei indicates that there may have been a shift in population structure, with modern strains lacking genomic islands that might encode virulence factors (50). An alternative explanation is that there have been changes in the host’s natural defenses, although the mechanism of this would be hard to explain in the absence of an adaptive immunity system in corals.

A further aspect of the V. shilonii-O. patagonica interaction was the apparent transformation into a viable but nonculturable (VBNC) state. The bacteria were reported to penetrate coral tissue and survive intracellularly (assessed using viable counts in the presence of gentamicin, which kills noninternalized bacteria), but that they are transformed into the VBNC form that multiplies within the coral tissue (assessed using total counts with a Live/Dead viability kit) but cannot be cultured on laboratory media (51). Large numbers of V. shilonii were also shown to survive in the VBNC state within the marine fireworm, Hermodice carunculata, which was proposed as a winter reservoir and vector for V. shilonii, responsible for reinfection of the coral each spring (52). Many vibrios are known to enter such a VBNC state (53), but the finding that this occurs as a result of intracellular growth (rather than starvation or low temperature shock) is very unusual. In our laboratory, we have shown that a typical VBNC state in V. shilonii can be induced in vitro at low temperatures and suggest that viable cells could successfully persist in seawater for significant periods during the lower temperatures that may be experienced in winter conditions (54). Unfortunately, there appears to be no further studies on the presence of V. shilonii in the fireworm, H. carunculata. It would be very interesting to see whether V. shilonii, V. mediterranei, or other related strains are still associated with the fireworm in the region and to compare their phenotypic and genomic properties with archived strains of V. shilonii in culture collections.

Finally, it is worth noting that seasonal bleaching of O. patagonica still occurs in the eastern Mediterranean and this fact has been used to claim that there is no apparent microbial influence over patterns of coral bleaching and to question the evidence that V. shilonii was ever the primary causative agent of bleaching (45). However, Rosenberg and colleagues provide strong counter-arguments that these claims are not justified based on evidence presented (55). Interestingly, Rubio-Portillo and coworkers (56) found that O. patagonica in the western Mediterranean showed a complex and dynamic community of Vibrio spp., which varied according to environmental conditions and coral health. They confirmed the presence of V. mediterranei only in diseased corals and showed that it caused rapid onset of disease signs during experimental infection of O. patagonica at elevated temperatures (56).

**VIBRIO CORALLIILYTICUS AS A CAUSE OF BLEACHING AND TISSUE LYsis IN TROPICAL CORALS**

The role of bacteria in coral bleaching was given further support by the finding of a new species of Vibrio as the cause of temperature-dependent bleaching and tissue loss in Pocillopora damicornis (57). Following the original description of strain YB1 from the Indian Ocean near Zanzibar, several other strains were isolated from P. damicornis showing bleaching and necrosis in 2001 from the Eilat reef in the Red Sea; these were
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characterized as a new species named *V. coralliilyticus*, closely related to *V. tubiashii*, *V. nereis*, and *V. shilonii* (57). Experimental infection of *P. damicornis* in aquaria was shown to be strongly dependent on temperature; at 24°C to 25°C, corals became bleached after 15 to 20 days and showed a significant reduction in the number of zooxanthellae, whereas at 27°C to 29°C, the corals showed rapid lysis of the tissues and died within 15 days (58). A powerful metalloprotease was isolated and shown to cause rapid tissue damage at 27°C (58). Subsequently, *V. coralliilyticus* has been identified in many regions of the world and it has emerged as a possible causative agent of white syndrome (WS) (Fig. 2A) epizootics in corals throughout the Indo-Pacific (59, 60). It has also been isolated from diseased octocorals in the Caribbean (61), from gorgonian corals in the Mediterranean (13, 62), and from bivalve larvae in England and Brazil (57). Phenotypic and phylogenetic comparison of 13 different *V. coralliilyticus* strains from various geographic regions and hosts revealed a high genetic diversity, suggesting lack of clonal population structures and providing no evidence for the rapid spread of a pandemic strain as an explanation for the apparent recent emergence of WS (61).

Further detailed studies on the role of the metalloprotease were carried out on *V. coralliilyticus* strains isolated from corals demonstrating WS (59). Screening of isolates using a protease assay and with specific primers for a *Vibrio* family metalloprotease gene showed a significant correlation between the presence of disease signs and the ability to produce the enzyme (59). When the effects of bacterial supernatants from four strains containing the protease were tested on the photosynthetic activity of *Symbiodinium* (zooxanthellae) cultures—obtained from the coral *Montipora aequituberculosa* on a part of the Great Barrier Reef (GBR) where WS has been observed—it was found that photosystem II was rapidly inactivated, probably by interaction of the protease with a specific target in susceptible *Symbiodinium*. By contrast, there was only a minimal photoinactivation of *Symbiodinium* from the same species of coral from another part of the GBR, where WS had not been observed (63). A second bioassay system, employing *Acropora millepora* juvenile corals, was used to show that the protease caused photoactivation in susceptible live corals, followed by loss of *Symbiodinium*, spreading lesions and mortality (63). Despite the strong link between the protease and the development of disease signs, these authors emphasize that WS is a multifactorial disease and that expression of other virulence factors and host traits are important. A similar conclusion was reached from results of a separate study of WS, in which the related species *V. harveyi* was strongly associated with development of the syndrome in aquarium studies with various corals, but was not found in all diseased specimens (64).

Whole genome sequencing and subsequent analyses of two strains of *V. coralliilyticus* from different geographic regions—P1, isolated from the GBR (59) and Vc450, isolated near Zanzibar (57)—have shed further light on the diverse virulence mechanisms of this pathogen (65, 66). Comparison of the genomes shows many similarities, with gene distribution on two chromosomes typical of that observed in other vibrios, the presence of large plasmids, and a large proportion of shared genes. However, 12% of each genome is unique, including genes for important virulence factors and pathogenicity islands that could explain different physiological characteristics, suggesting that the two strains could be regarded as distinct ecotypes or subspecies (65). Strain P1 possesses genes for 17 metalloproteases or putative proteases, some of which were predicted to be cytoplasmic or membrane-bound and others to be secreted (66). The vcpA gene was investigated in more detail and was found to be very similar to the hemagglutinin/protease genes described as major virulence factors in other vibrios, including *V. cholerae*, *V. splendidus*, and *V. tubiashii*. A mutant strain, in which the vcpA gene was deleted, was tested for production of various enzymes and for pathogenicity against *Symbiodinium* and two model animal systems (*Drosophila* and *Artemia*). Although the wild type and mutant strains showed marked differences in the enzyme profile and pattern of secreted proteins there was, surprisingly, no difference in pathogenicity. This led the authors to conclude that “the pathogenicity of vibrios in marine animals is a complex interplay of multiple genetic factors and unlikely the result of one determinant” (66). Some caution is perhaps necessary in the interpretation of such model systems for experimental determination of pathogenicity; for example, *V. coralliilyticus* and its extracellular products were previously shown to induce high mortalities of *Artemia* and fish, even when cultured at 18°C (68). In their study of the Vc450 genome, Kimes and coworkers (65) used a variety of bioassays and proteomic analysis to show that a number of virulence factors are upregulated at 27°C, correlating with the induction of bleaching and tissue damage previously observed with this strain (58). These include factors involved in motility, host degradation, secretion, antimicrobial resistance, and transcriptional regulation. Recent genome analysis of a *V. coralliilyticus* strain...
(OCN008) associated with white syndrome in *Montipora capitata* in Hawaii revealed similar virulence to those of other strains, but strain OCN008 did not cause temperature-dependent bleaching (68).

Boroujerdi and colleagues (69, 70) used nuclear magnetic resonance (NMR) to study the metabolomics profile of *V. coralliilyticus* during growth. They showed that there were significant changes between 24°C and 27°C in the levels of low molecular weight compounds involved in energy production and osmotic protection (especially betaine, succinate, and glutamate), but the significance of this is not clear (69). Motility and chemotaxis towards coral mucus appear to be especially important in colonization, as occurs in *V. shilonii* (42) and vibrios infecting other aquatic animals (71, 72, 73) and humans (74, 75). Meron and coworkers (76) used transposon mutagenesis to construct a mutation in the gene *flhA* of *V. coralliilyticus*. In chemotaxis capillary assays, the mutant showed almost no attraction to mucus from the coral *P. damicornis*, whilst in aquarium infection experiments the mutant was unable to attach to coral fragments and failed to initiate infection. Complementation of the mutation restored motility, attachment and infection (77). In the genomic study of *V. coralliilyticus* Vc450, genes for 57 chemotaxis proteins and 82 flagellar proteins were identified, with upregulation of methyl-accepting chemotaxis proteins and polar flagellar proteins at 27°C, lending support to the hypothesis that temperature dependent increases in motility and chemotaxis are important in virulence (66). Recently, *V. coralliilyticus* has been shown to use a newly described search pattern for chemotaxis (77), namely the “forward, reverse, flick, and repeat” behavior (78) first documented in *V. alginolyticus* (79). It was shown that *V. coralliilyticus* remained motile in both oxic and anoxic conditions—these occur within coral mucus in light and dark conditions, respectively—but that the “flick” search pattern is suppressed in anoxic environments, when the bacterium shows “run and reverse” chemotaxis (77). Garren and colleagues (80) used a novel microfluidics approach to show that *V. coralliilyticus* shows a strong chemotactic response to coral mucus, with the principal component responsible being the host metabolite dimethylsulfoniopropionate (DMSP). A correlation between heat stress and DMSP levels was shown and this enhanced the pathogen’s chemotactic response. As *V. coralliilyticus* did not appear to metabolize the DMSP, the authors concluded that the compound “is used purely as an infochemical for host location” (80).

**OTHER EVIDENCE OF VIBRIO DOMINANCE DURING DEVELOPMENT OF BLEACHING**

A disease termed yellow band/blotch disease (YBD) (Fig. 2B) has had a particularly damaging effect on the Caribbean reef-building coral genus *Montastrea* (81, 82).
YBD develops as pale yellow blotches on the coral tissue, which spread in a band as the disease progresses, with accompanying loss of zooxanthellae. Although the reduction in zooxanthellae was markedly affected by increased temperature between 26°C and 30°C, it was concluded that this bleaching had a different physiological basis to that associated with mass thermal bleaching events (83). Previous studies using culture-based methods implicated a consortium of *Vibrio* spp. (*V. rotiferianus*, *V. harveyi*, *V. alginolyticus*, and *V. proteolyticus*), which attack the zooxanthellae within the coral tissue of Montastrea and several other corals from both Caribbean and Indo-Pacific reefs, causing disruption of the algal cells in the field and in experimental infections (83, 84). Croquer and colleagues used DGGE and 16S rRNA gene analysis of clone libraries to examine healthy and YBD-affected corals, showing an increase in sequences of potential pathogens, especially *Vibrio* spp., although it was noted that apparently healthy corals also contained *V. harveyi* and that numbers of *V. harveyi* did not increase at the disease lesion interface (85).

It is important to understand the changes that might occur in coral-associated vibrios and other microbes, as these may provide an early indicator of environmental stressors and coral health. Indeed, the first speculation that *Vibrio* spp. might be implicated in bleaching came from the observation that these bacteria were not recovered from healthy specimens of Montastrea annularis, but constituted 30% of the bacteria isolated from bleached corals; the increased levels of vibrios returned to normal during recovery (86). Further studies demonstrated that *Vibrio* spp. populations tended to increase during bleaching of *M. annularis* but returned to previous levels during recovery, while populations of *Pseudomonas* spp. decreased during bleaching, but also returned to previous levels during recovery (87). In stressed *Acropora palmata* Caribbean corals, Ritchie (29) showed that *Vibrio* spp. dominated the heterotrophic bacteria cultured from apparently healthy colonies during a summer bleaching event, which was correlated with a loss in the antibiotic activity of the coral mucus and its associated resident microbiota.

In order to study changes in microbial community composition over time in corals on the GBR, Bourne and coworkers tagged specific *Acropora millepora* colonies and returned to collect samples at regular intervals over a 2.5 year period, which included a severe bleaching event, and analyzed them using clone library and DGGE analysis (88). As the temperature rose, the DGGE patterns and composition of the clone libraries changed to become dominated by *Vibrio*-affiliated sequences shortly before the visual signs of bleaching became apparent. The bacterial communities shifted again over a period of a few months as the coral colonies recovered from bleaching and regained their zooxanthellae (88). In a follow-up study, Littman and colleagues (89) studied juveniles of *Acropora tenuis*, which were heat stressed by raising the temperature from 28°C to 32°C. This study showed that the effects of thermal stress depend on complex interactions between different members of the coral holobiont – corals which harbored the type D clade of *Symbiodinium* zooxanthellae showed a dramatic shift towards vibrios, including *V. coralliilyticus*, which was not observed in corals containing clade C1 *Symbiodinium*. The shift in type D corals was accompanied by a 44% decline in photochemical efficiency; whereas type D corals that had been exposed to 30°C in the field showed a decrease in vibrios when transferred to the control temperature and the community changed to resemble that in the type C1 corals (90).

**COLONIZATION OF THE CORAL HOST BY VIBRIOS: DEFENSES AGAINST OXIDATIVE STRESS**

Because many corals contain photosynthetic zooxanthellae, coral tissue may contain exceptionally high levels of oxygen. Therefore, it is important to understand how vibrios and other coral-associated microbes cope with oxidative stress. Bacteria capable of colonizing healthy coral tissue would be expected to produce enzymes capable of overcoming the toxic effects of reactive oxygen species (ROS). Indeed, *V. shilonii* was shown to produce an extracellular superoxide dismutase (SOD) at 28°C, but not at 20°C, which was proposed to explain its ability to survive and multiply at the higher temperature, but to die below 20°C (91, 92, 93). Furthermore, a mutant deficient in SOD production was shown to be avirulent (90). A subsequent report (94) also concluded that SOD was a virulence factor for *V. shilonii* because the bacterium was more susceptible to external ROS at lower temperatures. Although this correlated with reduced SOD, no infection experiments were performed. Previously, in our laboratory, we had tested the effects of temperature, growth phase, and prior oxidative stress on the production of SOD and catalase in *V. shilonii* and a number of other vibrios isolated from corals (95). We observed significant differences in the activities of these enzymes in cultures, with upregulated expression of genes encoding these enzymes occurring with increased temperature. However, prior oxidative stress had no significant effect on...
the induction of catalase or SOD; we therefore concluded that these enzymes are unlikely to be major virulence factors and that their main function may be to protect against endogenous superoxide produced during growth (95). Further in situ investigations incorporating gene expression of bacterial and host factors during the diurnal cycle would provide valuable insight into the role of ROS in coral tissue and their influence on bacterial colonization.

**COLONIZATION OF THE CORAL HOST BY VIBRIOS: QUORUM SENSING**

As described in the previous section, shifts in coral-associated microbiota to populations dominated by vibrios are frequently observed. We know that in many other complex communities, quorum sensing (QS, the regulation of specific genes controlled by small signaling molecules at threshold population densities) is important in colonization, biofilm formation, and expression of extracellular enzymes and virulence factors (96). The mechanisms of QS have been particularly well studied in vibrios (97). The large diversity of vibrios and the different effects of temperature on production of QS signals and of virulence factors may explain the complexity of coral-associated community changes in response to environmental factors. Using a variety of biosensors for the detection of QS signal molecules, we screened numerous isolates of *V. campbellii*, *V. coralliilyticus*, *V. harveyi*, *V. shiloi*, *V. mediterranei*, *V. pelagius*, *V. rotiferianus*, *V. splendidus*, *V. tasmaniensis*, and *Photobacterium rosea* from a variety of healthy and diseased corals (98). Acyl homoserine lactones (AHLs) were found in over half of the vibrios tested, of which most produced a C4 acyl-chain-length signal molecule. We found considerable inter- and intraspecific variation and no correlation was observed between AHL production and the location of isolation (i.e., temperate versus tropical), the coral host, type of sample (i.e., water, tissue, or mucus) or whether the coral was diseased or healthy (98). Because temperature increases have been linked to increased colonization and virulence of vibrios for corals, we were particularly interested in the effects of temperature on both signal production and inhibition. In most cases, we found that the AHL profile was unaffected by temperature, but in some strains increased temperature inhibited production of short chain AHLs (98). Quorum sensing is known to control the production of enzymes in vibrios and so the relationship between enzyme activity (catalase, protease, and hemolysin) and temperature was also investigated for some strains. As expected, enzyme activity was generally higher at 25°C and 30°C than at 18°C, but apart from one strain of *V. harveyi*, there was no clear pattern between enzyme activity and AHL production for the other strains. This highlights the complexity of QS circuits in vibrios in which several systems often overlap to exert control of the production of enzymes (99). It is highly likely that more than one QS pathway is involved in the regulation of enzyme production in these coral-associated vibrios (98). This complexity was confirmed in the genome analysis of *V. coralliilyticus*, where four QS systems were identified (67). In a separate study of QS signals in bacteria isolated from different coral species, 81% of the *Vibrio* sp. isolates produced one or more AHLs, whilst others did not (notably including *V. coralliilyticus* and *V. shilonii*) (100). Whilst further in vitro studies on QS in cultured bacteria are important, it is likely that real progress in understanding the importance of this process in shaping coral bacterial communities will depend on the development of methods for detecting QS signals in situ.

**VIBRIO INFECTION IN TEMPERATE GORGONIAN CORALS**

Most of the research findings described above refer to diseases of tropical scleractinian corals, but disease associated with *Vibrio* spp. has also been described in gorgonian corals (sea fans) from temperate and cold waters. Mass mortality events in the gorgonian *Paramuricea clavata* have been linked to increased seawater temperatures in the Ligurian Sea (Mediterranean) in the summers of 1999 and 2003 (13, 63). Four potential pathogens were isolated and shown to reproduce the disease signs during experimental infection at 23°C, but not at 16°C; one of these isolates was identified as *V. coralliilyticus* (13). In a subsequent study, the seasonal variations of *Vibrio* spp. in seawater off the Ligurian coast were monitored, with a sharp increase in culturable vibrios observed above 22°C; *V. coralliilyticus* was only recovered at temperatures above 18°C (63). During an episode of mortality of *P. clavata*, consistently higher numbers of vibrios were recovered from diseased corals; healthy and diseased colonies had very different microbial communities. Of 48 vibrios isolated from diseased *P. clavata*, 15 were identified as *V. coralliilyticus*, 22 as *V. harveyi*, and 11 as *V. splendidus*, whereas, only *V. splendidus* was recovered from healthy corals. Representative isolates initiated temperature-dependent infection in aquarium corals, with *V. coralliilyticus* showing high virulence (63). In our laboratory, we have studied similar necrotic disease outbreaks in the closely related...
gorgonian *Eunicella verrucosa*, which occurred between 2003 and 2006 in the English Channel (Fig. 3). Of 21 distinct bacteria isolated from diseased tissues, 19 were vibrios presumptively identified as *V. splendidus* and *V. tasmaniensis* based on 16S rRNA gene sequencing (14), although further taxonomic characterization was not performed. In experimental infections, representative strains induced disease at 20°C, but not at 15°C, and we obtained preliminary evidence for the role of a protease in bacterial extracellular products in induction of tissue damage. However, we noted that the tissue samples examined in our study were often in an advanced state of necrosis and we were unable to sample zones immediately adjacent to the living tissue as the disease progresses. Therefore, it was not possible to ascertain whether vibrios are primary colonizers and a cause of the condition or whether they are a secondary effect (14).

**CONCLUSIONS**

Since the first description of vibrios associated with coral diseases in the 1990s, numerous studies have provided compelling evidence of the virulence of certain species for corals. Many of the signs of disease have been unequivocally attributed to extracellular products such as proteolytic enzymes and other factors. Some vibrios have been “proved” to be the causative agent of disease by fulfillment of Koch’s postulates, but experimental determination of pathogenicity in aquatic systems is fraught with difficulties and often employs unnatural conditions. Some prominent coral biologists have criticized the experimental basis for the claims of bacterial etiology (by vibrios and other species) for specific coral diseases (26). We should probably regard most—possibly all—vibrios as “opportunistic” pathogens that, under certain environmental conditions, are capable of overwhelming the defense mechanisms of appropriate hosts, leading to rapid growth and tissue destruction. Such mechanisms include intrinsic factors, adaptive immunity (if present) and the probiotic effects of resident microbiota. If there is a general lesson from this research, it is to recognize the fact that the great legacy of Koch, Pasteur, and the other founders of microbiology 150 years ago in discovering the causative agents of human bacterial diseases has, unwittingly, conditioned us to seek single microbes as the etiological agents of specific diseases. We should embrace the holobiont concept and recognize that many diseases in corals—as well as in plants, humans, and other animals—are multifactorial and depend on innumerable complex interactions between the host and all its microbial partners, profoundly affected by environmental influences.

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**REFERENCES**


The role of vibrios in diseases of corals


