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Bacterial Consortium of *Millepora dichotoma* Exhibiting Unusual Multifocal Lesion Event in the Gulf of Eilat, Red Sea

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Abstract Colonies of the hydrocoral *Millepora dichotoma* along the Gulf of Eilat are exhibiting unusual tissue lesions in the form of white spots. The emergence and rapid establishment of these multifocal tissue lesions was the first of its kind reported in this region. A characterization of this morphological anomaly revealed bleached tissues with a significant presence of bacteria in the tissue lesion area. To ascertain possible differences in microbial biota between the lesion area and non-affected tissues, we characterized the bacterial diversity in the two areas of these hydrocorals. Both culture-independent (molecular) and culture-dependent assays showed a shift in bacterial community structure between the healthy and affected tissues. Several 16S rRNA gene sequences retrieved from the affected tissues matched sequences of bacterial clones belonging to *Alphaproteobacteria* and *Bacteroidetes* members previously associated with various diseases in scleractinian corals.

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Introduction

The framework of coral reefs in the Red Sea is calcium carbonate-based and includes a highly biodiverse scleractinian coral assemblage. Milleporans are locally abundant hydrozoans that are important calcareous reef framework builders, second only to the Scleractinia (stony corals) [26]. The fire coral *Millepora* (family Milleporidae, class Hydrozoa, phylum Cnidaria) includes colonial, polypoid hydrozoans that secrete a calcareous skeleton [26]. Members of this genus occur worldwide in tropical seas and are among the most conspicuous of the skeleton-forming animals on coral reefs. In the Gulf of Eilat, Red Sea, Israel, the hydrozoan *Millepora dichotoma* is an important contributor to the reef, forming a calcareous base for reef growth as a distinct "*Millepora* zone" at depths of 0.2–3 m [28, 31].

As voracious plankton feeders, Milleporans also form a considerably important ecological component of the reefs [25]. Currently, *Millepora* sp. is considered a threatened genus and is listed by the International Union for Conservation of Nature due to extensive general reduction of coral reef habitat, but is also due to a combination of other threats, such as pollution, global warming, and disease [36].

In general, coral diseases have become more prevalent worldwide and have recently been reported to also affect the coral reefs of the Red Sea [1, 3, 41, 58]. Indeed, diseases, including biotically and abiotically driven syndromes, are cited as some of the main causes for coral reef decline [16–18]. Coral diseases known to be biotically driven include bacterial coral bleaching [23], Aspergillosis in sea

fans caused by the fungus *Aspergillus sydowii* [15, 49], white band and white plague diseases in Caribbean and Atlantic corals [54], and black band disease (BBD), a geographically widespread syndrome believed to be caused by a consortium of microorganisms [1, 4, 39]. Some of these disease syndromes are among the most important causes of coral deterioration in the Gulf of Eilat, followed by pollution and other anthropogenic interferences [29, 30]. In addition to diseases affecting scleractinian corals and gorgonians, there have also been a few reports recently describing diseases affecting calcareous hydrozoans [38, 57, 59].

In February 2010, Zvuloni et al., (2011) reported the occurrence of an unusual bleaching pattern in hydrozoan *M. dichotoma* populations in the Gulf of Eilat (Fig. 1a, b). They termed this lesion pattern "multifocal bleaching" (MFB) [57], as it was characterized by bleached spots scattered over the hydrocorals. More recently, Bruce A. Carlson from the Georgia Aquarium (personal communication) suggested that these white spots on *M. dichotoma* are feeding scars made by the leopard blenny, *Exallias brevis*. The frequency of MFB along the Eilat reef (Israel, northern tip of the Gulf of Eilat) was found to be extremely high, was much higher than that of any coral disease recorded in the region [59]. Owing to the unusually high frequency of this syndrome and to the lack of information available regarding the microbiota associated with the hydrocorals of this region, this study aims to characterize the bleached spots by distinguishing bacteria associated with the healthy and affected tissue of *M. dichotoma* as well as to characterize some of the tissue morphology associated with this hydrozoan syndrome.

Materials and Methods

Sample Collection

Fragments were collected from both the healthy and injured hydrozoans from the Eilat coral reef near the Inter-University Institute for Marine Science, Northern Red Sea (29°51′ N, 34° 94′ E) and transported immediately to the laboratory. Seawater samples were also collected from the surrounding reef area in sterile containers. In the laboratory, the live fragments were immediately subdivided into fragments for transmission electron microscope (TEM) examination and for microbial study.

Transmission Electron Microscopy

The fragments were enrobed in agar and fixed with 2.5 % glutaraldehyde in 0.22-μm filtered seawater for 1 h, then washed and gently mixed with 3 % bacteriological agar, and decalcified in EDTA. The samples were post-fixed with 1 % osmium tetroxide, dehydrated, embedded in araldite epoxy resin, and sectioned at 70–80 nm [19]. The resulting sections were stained with uranyl acetate and lead citrate and examined using a JEM-1230 transmission electron microscope at an 80-kV excitation voltage.

Microbial Studies

In the laboratory, the hydrozoan fragments collected for microbial examination were washed with sterile seawater, and tissue fragments weighing about 1 g were excised from both healthy and injured areas using a sterile scalpel. The excised hydrocoral fragments were crushed in 5 ml of sterile seawater using a sterile mortar and pestle to form slurry of tissues. The slurry from each fragment was treated as one sample and used for both total genomic DNA extraction and isolation of bacteria.

Total Genomic DNA Extraction

Total genomic DNA was extracted from the tissue slurry of the healthy and injured tissues and from the seawater sample using the MoBio Power Soil DNA isolation kit (MoBio Laboratories, Solana Beach, CA, USA). Genomic DNA was eluted using 60 μl of elution buffer or double-distilled water and stored at −20 °C. Concentrations were determined with an ND-1000 UV–Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

Isolation and Molecular Identification of Bacteria

The tissue slurry of the healthy and affected hydrocorals were serially diluted and plated on Marine Agar 2216 (HiMedia Laboratories, Mumbai, India) plates (10 % of

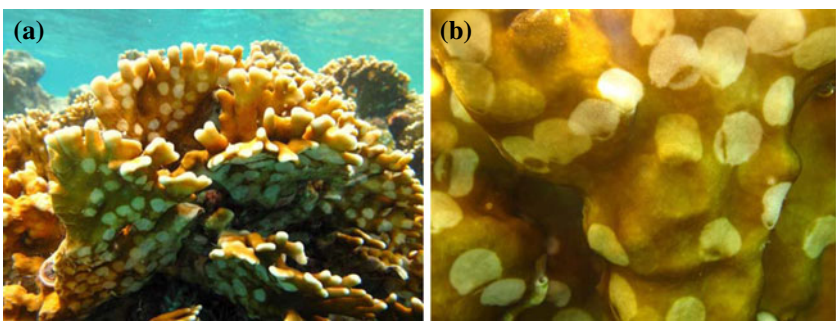

Figure 1 Affected *M. dichotoma*. (**a**) Prevalence of the multifocal lesion on the entire colony of *Millepora* sp. and (**b**) magnified image of the multifocal lesion

the recommended final concentration) for isolation of bacteria. The plates were incubated at room temperature (22–24 °C) for 1–2 weeks. After incubation, bacteria were randomly chosen and were grown as pure culture by repeated streaking on Marine Agar 2216 (50 % of the recommended final concentration) plates. The enumeration of culturable bacteria from the healthy and injured milleporan tissue is presented as CFU cm^{-2} of the hydrocoral surface [22]. Molecular identification of the cultured isolates was performed according to Shnit-Orland & Kushmaro (2009). Following incubation in 100 % marine broth, DNA was extracted from pure cultures using an UltraClean Microbial DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA) according to the manufacturer's instructions. DNA (20–100 ng μl^{-1}) was PCR-amplified and sequenced as mentioned above. Sequences were then compared with the GeneBank database (NCBI BLAST) and aligned using the MEGA program [55]. A phylogenetic tree was constructed by the neighbor-joining method [42].

PCR Amplification

Total DNA from healthy and injured tissues from the same coral colony were amplified using a Biometra TGradient thermocycler (Biometra, Gottingen, Germany), using the 16S rRNA gene universal primers 8F and 907R. The reaction mixture included 12.5 ml ReddyMix (ABgene, Surrey, UK), 1 μl of 10-mM concentrations of each primer (forward and reverse), 1 μl of 25-mM bovine serum albumin, 1–2 μl of the sample genomic DNA (environmental, 5–90 ng μl^{-1}), and water for a total volume to 25 μl. An initial denaturation hot start of 4 min at 95 °C was followed by 30 cycles of the following incubation pattern: 94 °C for 30 s, 54 °C for 30 s, and 72 °C for 1 min. For clone library production, a final extension at 72 °C for 30 min concluded the reaction.

Clone Library Construction and Sequencing

The heterologous 16S rRNA gene PCR products from the healthy as well as injured tissues were purified from the gel using the Wizard PCR Prep kit (Promega, Madison, WI), cloned into the pCRII-TOPO-TA cloning vector as specified by Invitrogen (Carlsbad, CA), and transformed into BioSuper $CaCl_2$-competent DH5α *Escherichia coli* cells (Bio-Lab, Israel) according to the manufacturer's instructions. Clones were examined for inserts by PCR amplification using M13-forward and reverse primers (Invitrogen), and DNA sequencing was performed using ABI PRISM dye terminator cycle sequencing ready reaction kit with AmpliTaq DNA polymerase FS and an ABI model 373A DNA sequencer (Perkin-Elmer).

Data Analysis

Sequences were first screened for chimeras using the Bellerophon server [20]. The classifier program at the RDPII site [9] was then used to assign sequences to a taxonomical hierarchy. Sequences showing <95 % homology were manually checked to ensure that corrupt sequences were eliminated. The remaining sequences from all clone libraries were aligned using MUSCLE [14], and a distance matrix was generated using the MEGA 4.0 software pack [55]. Sequences were assigned to operational taxonomic units (OTU) using DOTUR [43], and further analysis was carried out using SONS [44].

Nucleotide Sequence Accession Number

The 16S rRNA gene sequences isolated by culture-independent method from this study have been deposited in the NCBI GeneBank database under accession numbers HQ288552-HQ288737 (healthy and affected tissue of *M. dichotoma*), HQ288747-HQ288767 (seawater), and HQ288768-HQ288805 for the cultured strains.

Results

Microscopy

TEM revealed few bacteria in healthy tissue (Fig. 2a). Numerous bacteria were present in the interface between healthy and lesion tissues (Fig. 2c–e). Examination of the lesion tissues revealed general cellular necrosis and a loss of zooxanthellae (Fig. 2f) either via "symbiophagy" [13] or by the release of zooxanthellae from the tissues in comparison to a healthy *Symbiodinium* symbiosome (Fig. 2b).

Comparison of Bacterial Communities in Healthy and Affected Tissues

In the culture-independent study, the 16S rRNA gene clone libraries were constructed from the tissue of a healthy, non-affected *M. dichotoma* colony and compared with 16S rRNA gene clone libraries from affected colony. After elimination of chimeras, the healthy clone library consisted of 94 sequences, while the lesion clone library consisted of 92 sequences. The seawater clone library had 30 sequences. The clone library form the healthy tissue was populated mostly by *Gammaproteobacteria* (26 %), *Alphaproteobacteria* (22 %), *Sphingobacteria* (14 %), and *Firmicutes* (13 %) (Fig. 3a). On the other hand, the clone library from the affected tissues was mostly populated by *Alphaproteobacteria* (27 %), followed by *Sphingobacteria* (20 %) and *Gammaproteobacteria* (19 %) classes (Fig. 3b). *Spirochetes* were present in similar proportions in both clone libraries.

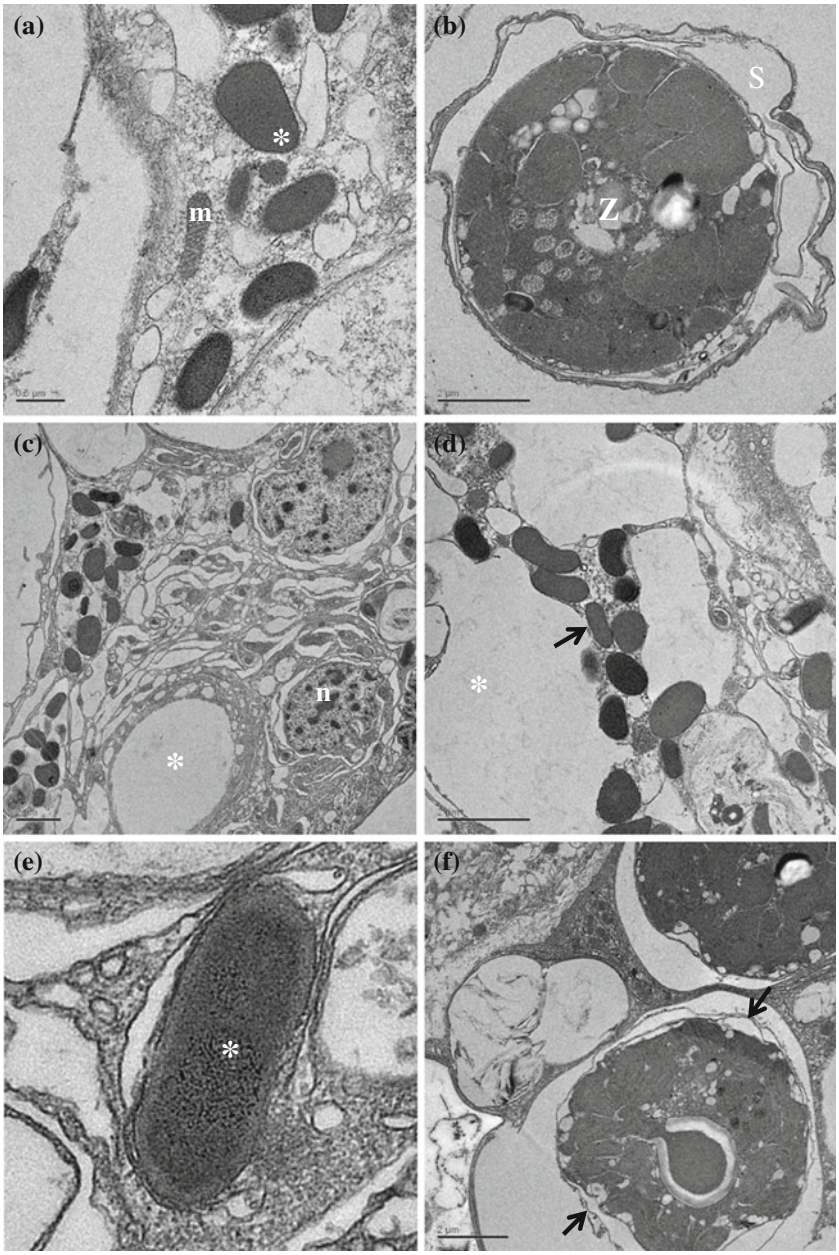

Figure 2 Transmission electron micrographs of *M. dichotoma*. (**a**) Apparently healthy tissue, note mitochondria (*m*) and lysozyme granules (*asterisk*); (**b**) Healthy *Symbiodinium* (*Z*) in symbiosome (*S*); (**c**) and (**d**) Interface between apparently non-affected tissue and affected tissues, characterized by the presence of healthy cells with visible nuclei (*n*), large vacuoles with fibril material (*asterisk*), granules and numerous bacteria (*arrow*); (**e**) Magnified image of a bacterium (*asterisk*) present in the affected tissue; (**f**) Affected tissue showing necrosis and evidence of symbiophagy of damaged zooxanthellae in symbiosome/phagosome as characterized by separation of *Symbiodinium* membranes from the cell (*arrows*)

The clone library of the surrounding seawater was dominated by *Cyanobacteria* (79 %), a group that was absent in both the healthy and injured tissue clone libraries (Fig. 3c).

The overall diversity of the cloned sequences (total $n=216$) was analyzed on two levels (≥90 and ≥97 % similarity) using cluster analysis by the DOTUR program [43]. The results are summarized in Table 1. A total of 28 OTUs were observed from the healthy tissue at the ≥97 % similarity level (roughly representing species level) compared with 25 OTUs obtained from the affected tissue. The results for the Chao1 richness estimator and Shannon–Weaver diversity index (Table 1) showed that the bacterial diversity was higher in the injured tissue when compared to that of the healthy tissue. A similar image was obtained for the ≥90 % similarity level (roughly representing order–class level) (Table 1). Lower diversity in healthy tissue indicates that a bacterial community is dominated by several species (OTUs), while injured tissue have more similar abundance (number of singletons) per OTU. This could suggest a more specialized community in the healthy tissue.

A Venn diagram illustrates the observed overlap of OTUs between libraries of the healthy and injured tissues and the eleven unique OTUs with no overlap formed in the seawater clone library (Fig. 3d). Among the healthy and damaged clone libraries, a total of 39 OTUs were detected. The healthy tissue and the affected tissue clone libraries consisted of 14 (36 %) and 11 (28 %) distinct OTUs, respectively. Fourteen OTUs overlapped between the healthy and damaged tissue clone libraries. The closest match of representative sequences

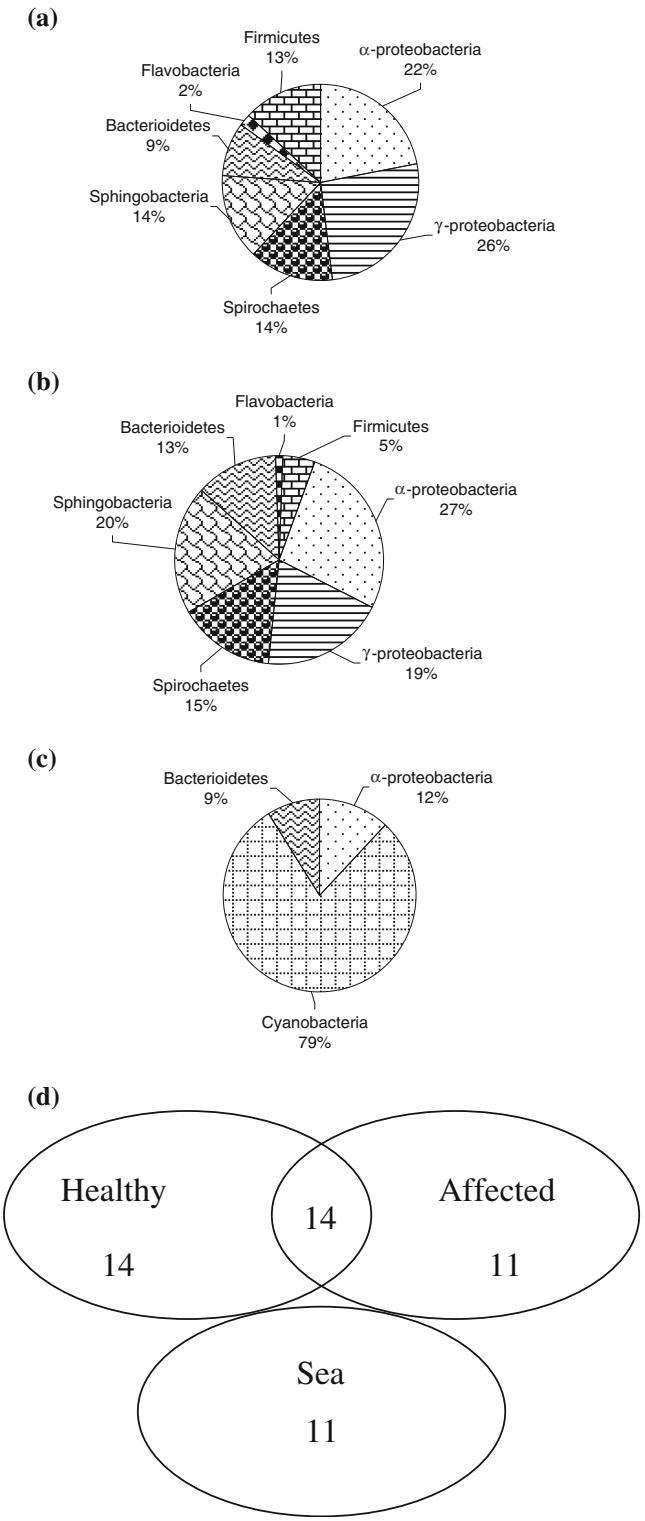

Figure 3 Division-level diversity of the 16S rRNA gene bacterial sequences obtained from clone libraries constructed with universal primers from *M. dichotoma*. (**a**) Healthy tissue, (**b**) affected tissue, and (**c**) seawater analyses and (**d**) a Venn diagram describing the overlap between the healthy tissue, affected tissue, and seawater clone libraries for the total dataset. The number in each segment (**d**) indicates the number of distinct OTUs present in the clone library. The numbers in overlapping segments indicate the number of OTUs shared between the healthy and damaged tissue libraries from each OTU of the healthy and damaged clone libraries are shown in Fig. 4.

Among the 11 distinct OTUs of the injured tissue-associated microorganism clone library, several sequences matched those of bacterial clones associated with various diseases in corals. Clones D108 (HQ288733) and D45 (HQ288680) showed 99 % sequence similarity to uncultured *Alphaproteobacterium* sequences BBD 217 15 (DQ446102) and BBD 216 07 (DQ446131), respectively (Fig. 4), which were retrieved from BBD-infected tissue of the coral *Siderastrea siderea* [47]. Clone D35 (HQ288672) displayed 91 % similarity to the 16S rRNA sequence SBBAC2_A04 (JF835682) retrieved from a bleached colony of *Siderastrea stellata*, whereas clone D113 (HQ288737) was similar (97 %) to Acer_M02 (GU117970) retrieved from the coral *Acropora cervicornis* [51] and at 92 % homologous to uncultured *Kordiimonas* sp. (DQ917810) isolated from the bleached coral *Muricea elongata* (Fig. 4). Among the sequences that clustered with *Bacteroidetes*, the clone D76 (HQ288709) was closely related (97 %) to an uncultured bacterium clone Mfav_O11 (GU118661) isolated from *Montastraea faveolata* coral [51] and distantly related (91 %) to an uncultured bacterium clone SBBAC_B01 (JF835618) retrieved from bleached colonies of the coral *S. stellata* (Fig. 4). Most of the sequences (79 %) in the seawater clone library (Fig. 3c) were very closely matched (≥98 % sequence similarity) to uncultured cyanobacterial sequences retrieved from seawater in other geographically distant areas.

Isolation and Identification of Bacterial Communities

The culturable bacterial counts in the healthy and injured hydrocoral tissue were $5.6\times10^5\pm7\times10^4$ CFU cm^{-2} and $8.0\times10^6\pm3\times10^3$ CFU cm^{-2}, respectively. A total of 37 bacterial strains were isolated from *M. dichotoma*. Twelve isolates were obtained from the healthy tissue and 25 from the injured tissue. The strains were characterized using 16S rRNA gene sequencing. The culturable bacterial diversity between the healthy and lesion tissues showed marked variation. A higher number of bacterial diversity was noticed in the lesion tissue. The healthy tissue was strongly dominated by G*ammaproteobacteria* (82 %) followed by *Firmicutes* (18 %), both of which were less abundant (19 % and 5 %, respectively) in the injured tissue. The dominant bacteria in the injured tissue comprised *Alphaproteobacteria* (42 %), *Actinobacteria* (29 %), and *Flavobacteria* (5 %); all of which were totally absent in the healthy tissue.

Phylogenetic analysis of the cultured isolates from the healthy tissue showed that more than 60 % of the 16S rRNA gene sequences of the *Gammaproteobacteria* belonged to the genus *Vibrio*. The isolates MH1 (HQ288768), MH5 (HQ288771), and MH6 (HQ288772) were closely related (≥99 % similarity) to the *Vibrio* sp. (GQ406785;

Table 1 Number of OTUs and richness estimation of 16S rRNA gene libraries from healthy and affected tissues of *M. dichotoma*

16S rRNA gene clone library	No. of clones sequenced	Richness estimators					
		No. of OTUs		Chao1		Shannon–Weaver Index	
		Cutoff 97 %	Cutoff 90 %	Cutoff 97 %	Cutoff 90 %	Cutoff 97 %	Cutoff 90 %
Healthy	94	28	18	33	21	2.54	2.35
Bleached	92	25	15	41	22	2.68	2.47

Shannon–Weaver diversity index and Chao1 richness estimator were computed using DOTUR. Numbers of OTUs, Chao1 estimated richness, and Shannon–Weaver diversity index are shown for both 3 and 10 % differences in nucleic acid sequence alignments

Pseudopterogorgia americana), *Vibrio tubiashii* (NR026129), and *Vibrio fortis* (AB470938; *Montipora* sp.), respectively. Strains MH4 (HQ288770), MH14 (HQ288778), and MH15 (HQ288779) were related to *Vibrio* sp. isolated from various aquaculture organisms.

Phylogenetic analysis of the cultured isolates from the affected tissue showed that the isolate MD2 (HQ288781), and the D113 sequence (Fig. 4) retrieved from our tissue affected clone library, had 98 % similarity to an uncultured bacterium clone Acer M02 (GU117970) from the coral *A. cervicornis*. Isolate MD2 was distantly related (91 %) to an uncultured *Kordiimonas* sp. clone BME88 (DQ917810) belonging to *Alphaproteobacteria* isolated from the injured coral *M. elongata*. The isolate MD21 (HQ288792) displayed 99 % similarity to the 16S rRNA sequences of uncultured alpha proteobacteria AM930440 and AM930441 isolated from body wall lesions of the sea urchin *Tripneustes gratilla* [5], of FJ203221 and FJ203273 isolated from white plague diseased tissue of *M. faveolata* [50], and of EF123405, DQ446157, and DQ446160 retrieved from BBD tissues of *S. siderea* [45, 47]. The isolate MD32 (HQ288802) displayed 95 % similarity to FJ203204, FJ203272, FJ203275, and FJ203299 isolated from bleached *M. faveolata* coral [50]. The isolate MD35 (HQ288805) displayed 99 % similarity to *V. fortis* (HM584115) isolated from puffer fish skin lesion, to *Vibrio* sp. HB-8 (AY876051) isolated from brown band syndrome of the *Acropora muricata* coral, and to uncultured bacterium (EF089442) from mucus layer adjacent to the BBD mat of *Favites* sp. [4].

Discussion

The study showed that there was a shift in the bacterial OTUs between the healthy and injured tissues of *M. dichotoma*. Interestingly, *Alphaproteobacteria*, *Gammaproteobacteria,* and *Sphingobacteria* constituted significant proportions of the clone libraries of both healthy and affected tissues (Fig. 3), indicating that bacteria belonging to these classes may be part of the general normal flora of the hydrozoan tissue. Despite this, there were evident differences in bacterial OTUs of the *Alphaproteobacteria* and *Bacteroidetes* communities (Fig. 4) between the affected and healthy tissue. The culture-independent study showed that the microbial community in the seawater clone library was totally different from both the healthy and injured tissue clone libraries (Fig. 3), indicating that these microorganisms were not part of the general seawater flora.

Most of the sequences of the 14 distinct OTUs of the healthy hydrozoan tissue clone library (Fig. 3d) matched sequences of clones retrieved form healthy tissues of scleractinian corals and sponges (Fig. 4). This may indicate that these bacteria are either commensals or mutualistic symbionts of these hydrozoans, similar to what was reported for corals [33]. It is further possible that these microorganisms may play a "probiotic role" within the hydrozoan holobiont [34].

Among the 11 OTUs retrieved from the affected tissue, several were closely associated with members of the group of *Alphaproteobacteria* associated with diseased scleractinian corals such as BBD in *S. siderea* [46, 47], *M. faveolata* [50], *Diploria strigosa*, and *A. cervicornis* [51]. Members of the *Alphaproteobacteria* group are widely associated with diseased scleractinian corals and have been reported to be responsible for causing numerous disease symptoms in corals [7, 12]. Interestingly, a species of this group, a new *Sphingomonas* sp., was found to be associated with a form of white band or "white plague" syndrome on *Millepora* sp. in Florida [38]. Furthermore, an *Alphaproteobacteria* closely related to the causative agent of juvenile oyster disease (AF114484) was also found to cause white plague-like disease in the Caribbean coral *Montastrea annularis* [37]. The same ribotype was also found in the bacterial consortium associated with BBD in the scleractinian coral *Colpophyllia natans* [11]. Indeed in the Red Sea, *Alphaproteobacteria* were the second most dominant member in the BBD-infected corals of the genus *Favia* [1]. An additional Alphaproteobacteria, *Aurantimonas coralicida*, a novel bacterium belonging to an unknown marine taxon of the order *Rhizobiales*, was documented as the causative agent of white plague Type-II disease in the scleractinian coral *Dichocoenia stokesi* [12]. These results indicate that members of the *Alphaproteobacteria* implicated in a number of scleractinian coral diseases are also present in the affected tissue of *M. dichotoma*,

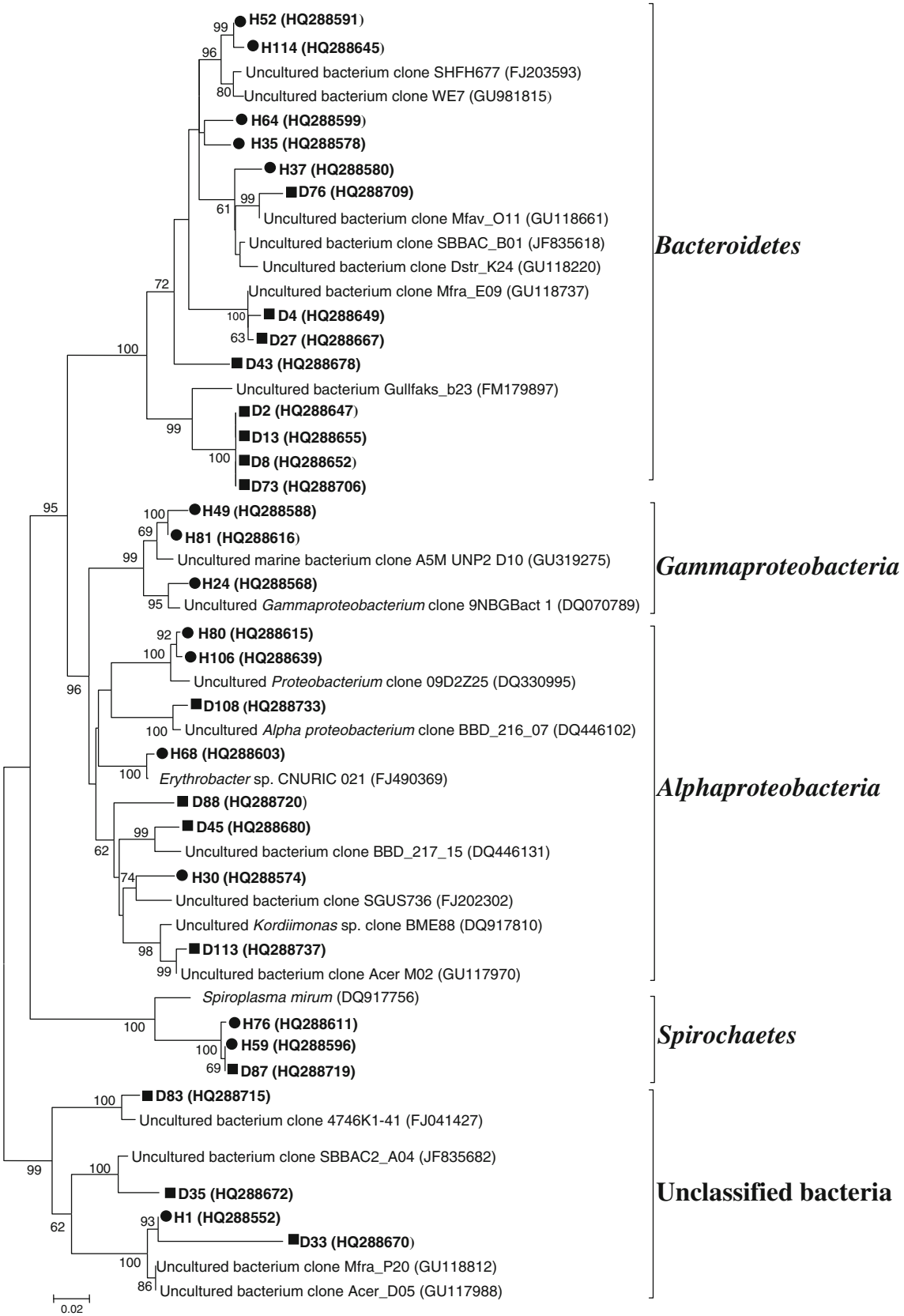

Figure 4 Neighbor-joining phylogenetic tree of representative clones from each OTU of the healthy (*filled circle*) and affected (*filled square*) tissue of *M. dichotoma* and the closely related sequences obtained from the GenBank. The *bar* represents two substitutions per 100 nucleotide positions. Bootstrap values are indicated at *branch nodes*

indicating that this group of bacteria may also play a role in the milleporan disease, though this merits further study.

In the present study, the bacterial count was high in affected compared to healthy tissue. Likewise, Chao1 and Shannon indices (Table 1) indicated an increase in diversity at the phylum–order and genus–species levels in the affected tissue when compared with the healthy tissue. This is in agreement to previous studies of corals [52] and sponges [32], and it has been hypothesized that the increased bacterial diversity in diseased animals may be due to the elevation in nutrients associated with decaying cells and/or a breakdown in the host defense mechanisms [56]. In contrast to what was found using culture-based techniques, the molecular study of the bacteria associated with healthy fire coral tissues showed a high number of *Alphaproteobacteria* (Fig. 3a), underscoring the importance of using multiple techniques to assess bacterial diversity.

In the healthy Millepora tissue, *Vibrio* was the dominant genus from the group of *Gammaproteobacteria*. It is possible that some *Vibrio* spp. may establish mutualistic partnerships with these hydrocorals and provide them with nutrients and secondary metabolites (e.g., bacteriocins) [40]. Indeed, recent studies showed that *Vibrio* spp. associated with the coral mucus of several corals produced antibacterial compounds against several pathogens, thereby may aid in the protection of the coral host against pathogens [35, 48]. On the other hand, some *Vibrio* spp. are also known as coral obligatory or opportunistic pathogens [6, 23, 53] and their proliferation have the ability to introduce substantial changes in the coral microbial community structure that are potentially linked to their antibacterial activity which inhibit growth of other bacterial ribotypes [24]. Similar to the culture-independent study, the pervasiveness of *Alphaproteobacteria* members through culture-dependent study further proves that there is a definite shift in the bacterial population between the healthy and affected tissue, and some of the isolates closely resembled bacterial strains that were isolated in stressed corals [22].

It is difficult to pinpoint a causative microbial agent responsible for the change in tissue morphology, and therefore, the possibility that the disease-like syndrome is of environmental origin should also be taken into account. Similar kinds of syndromes have been shown to occur in sponges as a result of various extrinsic factors, including physical damage [8] and predation [21], and due to the chemical defense mechanisms mediated by sponges against the invading pathogen [27]. Bleaching due to extrinsic factors such as elevated seawater temperature has also been observed in *Millepora* sp. as reported by Cook et al., (1990) for *Millepora alcicornis* in Bermuda in 1988. The bleaching patterns in these organisms though were not microbially defined. In the present paper, we attempted to further clarify this phenomenon by characterizing the microbial population in affected and healthy tissues.

In this study, we also demonstrated that although the syndrome's gross morphology was characterized by seemingly bleached, confluent, and intact tissues, microscopic examination revealed that the affected areas actually underwent some tissue necrosis. In conclusion, a clear shift in microbial population was evident between the healthy and affected tissues, and the affected tissues showed marked necrosis, zooxanthellae release, and symbiophagy [13], whereupon the zooxanthellae were digested by the host (Fig. 2). In addition, we showed that the affected tissue was populated by members of *Alphaproteobacteria* that are closely related to isolates from other diseased corals. Though disease-like syndromes in Milleporans have previously been reported in other geographic areas [2, 10], the present study is the first to describe the bacteria associated with the healthy and affected tissue of this hydrozoan syndrome. Further studies on this hitherto unreported disease involving different environmental factors and additional investigation into microbial changes occurring during this "disease" process may eventually reveal a conclusive cause for the occurrence of this multifocal lesion event.

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