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Bacterial communities of juvenile corals infected with different *Symbiodinium* (dinoflagellate) clades

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ABSTRACT: The coral holobiont consists of the host and its microbial partners, including the dinoflagellate endosymbiont *Symbiodinium* and bacteria living both on and within coral tissues. Although genetically different, *Symbiodinium* types have been shown to differentially affect the physiology of the coral host; their effects on the bacterial partners in the association are unknown. The present study compares profiles of the bacterial communities associated with juvenile corals of *Acropora millepora* and *A. tenuis* that had been experimentally infected with 2 different clades of *Symbiodinium*, Clade C1 and D, to investigate possible interactions between bacterial and *Symbiodinium* communities. Three culture-independent 16S rRNA gene profiling methods (clone library construction, terminal restriction length polymorphism and denaturing gradient gel electrophoresis) revealed no discernible pattern in bacterial communities on 9 mo old juvenile corals containing different clades of zooxanthellae, suggesting that coral-associated bacteria are not linked to *Symbiodinium* types *in hospite* in early ontogeny. In contrast to bacterial profiles of adult corals, bacterial communities associated with juvenile corals were highly variable, indicating that bacterial associates are not conserved in these early stages. When 12 mo old juveniles were sampled again in summer, bacterial communities associated with *A. tenuis* hosting Clade D *Symbiodinium* were dominated by sequences affiliating with *Vibrio* species, indicating that corals harbouring this symbiont may be more susceptible to temperature stress, allowing growth of opportunistic microbial community members possibly detrimental to coral health.

KEY WORDS: *Symbiodinium* · Bacteria · Coral · Holobiont · *Vibrio*

INTRODUCTION

Reef-building corals host a variety of microorganisms, including symbiotic dinoflagellates called *Symbiodinium* (zooxanthellae) and an array of *Bacteria*, *Archaea* and viruses (reviewed in Knowlton & Rohwer 2003). For decades, research on coral symbioses has focused on *Symbiodinium* and its photosynthesis, revealing the important roles that the algal endosymbiont plays in providing a source of carbon necessary for coral growth and in assisting calcification (Barnes & Chalker 1990, Muller Parker & D'Elia 1997). In contrast, the roles of bacterial and archaeal associates that populate the mucus and tissue layers of corals are poorly understood. Potential benefits for the coral holobiont (which includes the host and all microbial partners) can be inferred from bacterial isolates and affiliated sequences retrieved from previous studies investigating coral-associated bacteria. Some coral-associated bacteria are known to fix nitrogen and carbon and may pass products to the coral host (Williams et al. 1987, Shashar et al. 1994, Cooney et al. 2002, Rohwer et al. 2002, Lesser et al. 2004). Other bacteria have been found to produce secondary metabolites such as antibiotics (Castillo et al. 2001). Moreover, bacteria isolated from the mucus of healthy *Acropora palmata* have been shown to inhibit growth of potentially pathogenic microbes (Ritchie 2006). Thus, bacterial communities may play important roles in maintaining coral health.

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Recently, the role and diversity of algal and bacterial symbionts, as well as the possibility that corals may adapt to environmental conditions by altering their symbiont communities (Buddemeier & Fautin 1993, Reshef et al. 2006) have been the focus of a number of studies. It has been shown that different genetic types of *Symbiodinium* can differentially affect the physiology of the coral host. For example, corals containing *Symbiodinium* Clade D tend to be more tolerant to heat stress (Baker et al. 2004, Berkelmans & van Oppen 2006), although species-specific coral–*Symbiodinium* interactions may modify this general pattern (Abrego et al. 2008). Furthermore, both *Acropora tenuis* and *A. millepora* juveniles grow 2 to 3 times faster when associating with *Symbiodinium* Clade C1 compared to those associated with Clade D (Little et al. 2004). Faster growth of corals infected with *Symbiodinium* C1 may reflect a greater contribution of the symbiont to host nutrition through faster rates of population growth inside the host (Fitt 1985) and/or doubling of the rate of photosynthate translocation to host tissues (Cantin et al. 2009). However, to date, no studies have investigated how different symbiotic *Symbiodinium* clades within the coral host might affect other microbial partners in the coral holobiont. As a multispecies mutualism, the relationship between corals and their symbionts may have an additional dimension; *Symbiodinium* endosymbionts may interact differentially with bacterial associates competing for space within the host to shape aspects of holobiont physiology. For instance, most (as high as 98%) of the net carbon assimilated by *Symbiodinium* is released as exudates, much of it excreted into the mucus (Ikeda & Miyachi 1995). Mucus composition is potentially important in structuring microbial communities (Ritchie & Smith 2004). Ritchie & Smith (1997) demonstrated that selective carbon source utilization by cultured bacteria differs among 11 coral species. Variation in photosynthetic contributions by different *Symbiodinium* could therefore affect the composition of coral mucus, indirectly impacting the coral microbiota. Changes in algal symbionts associated with corals may lead to differences in the bacterial populations on corals, and this may have larger implications in times of environmental stress, as changes in one component of the microbial community may cause a complete shift in coral-associated microbial communities.

To further our understanding of possible interactions between bacterial and *Symbiodinium* communities associated with coral, the present study compared the bacterial community profiles of 9 mo old juvenile colonies of the corals, *Acropora millepora* and *A. tenuis*, which had been experimentally infected with 2 different clades of *Symbiodinium*, C1 and D. Juvenile corals were raised from larvae to enable manipulation of the *Symbiodinium* type harboured upon initiation of symbiosis. Bacterial profiles of juvenile corals were compared to those of adult corals containing the same clade of *Symbiodinium* to determine if age affects the composition of bacterial communities. A second sample of *A. tenuis* juveniles (12 mo old) containing Clades C1 and D collected 3 mo later in summer was analysed to determine whether temporal changes, such as increase in temperature, might differentially influence bacterial communities associated with corals harbouring different *Symbiodinium* types. Three culture-independent profiling methods were used to analyse bacterial associates: denaturing gradient gel electrophoresis (DGGE), clone library construction, and terminal restriction fragment length polymorphism (tRFLP) to cross-validate findings.

MATERIALS AND METHODS

Gamete collection, larval settlement and *Symbiodinium* infection. Juveniles of the corals *Acropora tenuis* and *A. millepora* were produced and experimentally infected with *Symbiodinium* C1 or D as described in Abrego et al. (2008). In brief, adult colonies of both species were collected from the upper reef slope in Nelly Bay, Magnetic Island (19° 10′ S, 146° 50′ E) just prior to spawning in November 2006 and placed in separate tanks onshore. Following spawning, gametes were collected from 4 colonies of each species and mixed in aquaria containing 1 µm filtered seawater to effect fertilization. Once cleavage was detected, embryos were moved into larger culture tanks supplied with flow-through 1 µm filtered seawater throughout larval development. When larvae commenced searching for settlement sites, sterilized terracotta tiles were placed on the bottom of culture tanks to provide settlement surfaces. Autoclaved coralline algae were then added to provide settlement cues for larvae. Following settlement, tiles were divided into separate tanks for infection with algal endosymbionts.

Settled *Acropora tenuis* and *A. millepora* corals were experimentally infected with both homologous and heterologous *Symbiodinium* clades. Algal isolates were prepared by airbrushing adult colonies of: (1) *A. tenuis* to collect *Symbiodinium* C1 and (2) *A. millepora* to collect *Symbiodinium* D. Colonies were collected from Magnetic Island, where these species are known to be associated with the targeted *Symbiodinium* clades (Little et al. 2004). Tissue slurries were centrifuged (3000 × *g*, 3 min) to pellet *Symbiodinium* cells, and liquid was removed. Pellets were re-suspended in freshly filtered seawater and repeatedly washed until coral tissue was removed. Algal cells were counted under an Olympus BH-2 light microscope (Olympus Corp.) using a haemocytometer to determine concen-

trations of cells. Isolates were diluted to approximately the same density of *Symbiodinium* cells (about 400 cells ml^{-1}) and added in equal amounts into tanks containing settled larvae.

Following infection with the appropriate algal symbiont, colonies were sampled and the *Symbiodinium* clade was verified by single-stranded conformation polymorphism (SSCP). At the approximate size of 3 to 5 polyps, juvenile corals were returned to the parental habitat, where they remained until sampled. Tiles containing juvenile corals were placed on steel rods and suspended horizontally between pairs of metal star-pickets on the reef flat in Nelly Bay, Magnetic Island.

Sample collection and processing. 9 mo (September 2007) and 12 mo (December 2007) after *Acropora tenuis* and *A. millepora* juveniles were out-planted to the reef, 6 juveniles containing *Symbiodinium* C1 and 6 containing *Symbiodinium* D from each coral species (i.e. 24 juveniles in total at each sample time) were removed from the settlement tiles by detaching colonies with a sterile scalpel (Table 1). Water temperatures were 25°C in September and reached 30°C in December. Whole juvenile colonies were rinsed with artificial seawater, placed in cryovials and stored at –80°C. The number of surviving juveniles was counted, and the size of each colony was measured at each sample time.

DNA extraction and purification. DNA was extracted by suspending an entire juvenile colony in 0.5 ml of buffer (0.75 M sucrose, 40 mM EDTA, 50 mM Tris, pH 8.3) and following the extraction protocol outlined previously by Bourne et al. (2008). The DNA pellet was suspended in 30 µl of sterile milli-Q water, and the total volume was loaded on a 1.2% low-melting agarose gel. DNA was purified by using electrophoresis and cutting high-quality DNA (>2 kb) from the gel.

Table 1. *Acropora millepora* and *A. tenuis*. Sampling design and survey results. N: number of replicates; NA: not applicable

Age (mo)	*Symbiodinium* clade	N	Size (cm)	Alive (n)
A. tenuis				
9	C1	6	1–2	126
	D	6	0.5–1	81
12	C1	6	2–3	112
	D	6	1	18
Adult[a]	C1	3	NA	NA
A. millepora				
9	C1	6	1–2	123
	D	6	0.5–1	63
Adult[a]	D	3	NA	NA

[a]Sample data from Littman et al. (2009)

The agarose was then removed from the sample by using the QIAquick gel extraction kit (Qiagen), following the manufacturer's instructions. DNA was recovered from the Qiagen column with two 30 µl washes of sterile milli-Q water.

PCR amplification of the 16S ribosomal RNA gene. Universal primers 63F and 1387R (Marchesi et al. 1998) were used to amplify the 16S rRNA genes from extracted DNA for bacterial clone library construction. Amplification of the 16S rRNA genes for tRFLP analysis was performed using the D4 labelled 63F primer and 1389R primer. The PCR mixtures (50 µl) contained 0.2 pmol $µl^{-1}$ of each primer, 200 µM each deoxynucleoside triphosphate, 1× PCR buffer [Tris-Cl, KCl, $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$], 0.08% (w/v) bovine serum albumin and 1.25 U of *Taq* polymerase (Scientifix). PCR was performed with an Applied Biosystems 2720 thermocycler and programmed with an initial 3 min step at 94°C and 35 cycles consisting of 94°C for 1 min, 55°C for 1 min and 72°C for 1.5 min and a final extension for 10 min at 72°C.

For DGGE, the bacterial 16S rRNA gene was amplified using primers 1055F and 1392R-GC (Ferris et al. 1996). PCR reactions (50 µl) consisted of 0.5 µM of each primer, 100 µM of each deoxyribonucleotide triphosphate, 0.08% (w/v) bovine serum albumin, 1× PCR buffer [Tris-Cl, KCl, $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$], 1.5 mM $MgCl_2$ and 1.25 U of Hotstar *Taq* (Qiagen). DGGE PCR reactions were carried out using an Eppendorf Mastercycler thermocycler. Temperature cycling was performed using a touchdown protocol (Ferris et al. 1996) with 1 cycle of 95°C for 15 min, 10 cycles of 94°C for 1 min, 53°C (each cycle decreasing by 1°C) for 1 min and 72°C for 1 min, followed by 20 cycles of 94°C for 1 min, 43°C for 1 min and 72°C for 1 min.

SSCP. SSCP was used to identify the *Symbiodinium* type present in each juvenile at each sampling to ensure that corals still harboured the same C1 or D *Symbiodinium* type with which they were initially infected. The 18S internal transcribed spacer 1 (ITS1) region of *Symbiodinium* clades was amplified using ITSF and ITSR primers (van Oppen et al. 2001). PCR products were diluted 1:3 with formamide dye and denatured by placing them at 95°C for 5 min and then immediately on ice. Products were loaded on 4% acrylamide gel and run on a Gel-Scan 3000 (Corbett Robotics) with 0.5× TBE buffer (0.01 M Tris base, 0.01 M boric acid and 0.2 mM Na_2 EDTA; pH adjusted to 8.3). Temperature was set at 22°C and run at 1200 V for 25 min. Gels were imaged using Gel-Scan 3000 software (Corbett Robotics).

Clone library construction. The amplified bacterial DNA was ligated into TOPO-cloning vector following the manufacturer's instructions (Invitrogen). Ligations

were submitted to the Australian Genome Research Facility for transformation, cloning and subsequent sequencing. Two replicate libraries of 96 clones each were sequenced for samples containing each *Symbiodinium* clade for both *Acropora* species. Dominant bacterial 16S rRNA gene sequences retrieved from each clone library were analysed using principal components analysis (PCA) to determine which sequences (affiliated at the genera level) were important in driving differences between the libraries. Due to the high amount of variability in each library, only 16S rRNA gene sequences that constituted 2% or more of any clone library were included in the PCA analysis.

DGGE. Bacterial profiling was carried out using an INGENY phorU-2 (Ingeny International BV) DGGE system. PCR products were separated on gels containing 6.5% acrylamide with a 50 to 70% linear gradient of formamide and urea, using 0.5× TAE buffer (0.02 M Tris base, 0.01 M sodium acetate and 0.5 mM Na_2 EDTA; pH adjusted to 7.4). The buffer was preheated to 60°C prior to sample loading, and the electrophoresis was run at 30 V for 20 min to draw the DNA into the gel before running buffer through the system. Electrophoresis was then run at 60°C for 16 h at 70 V. Gels were removed and stained for 10 min with SYBR Gold nucleic acid stain (Molecular Probes Inc.) in 1× TAE buffer. Gels were de-stained by rinsing with 1× TAE buffer and subsequently photographed using a trans-illuminator.

Clear bands were excised from the gel and placed in 100 µl of sterile milli-Q water to elute DNA from the acrylamide gel. The DNA bands were re-amplified and run on the DGGE gel to ensure correct migration and purity of the product. Products that showed 1 distinct band with the correct mobility on the DGGE were directly sequenced. Using the sequences recovered from cut bands, a presence/absence matrix was constructed for the DGGE fingerprints and analysed by non-metric multi-dimensional scaling (nMDS) using Euclidian distances.

tRFLP. Prior to restriction digestion, 3 replicate PCR products for each sample were pooled and purified using the QIAquik PCR Purification Kit (Qiagen). The restriction reaction mix was prepared with 1× NEB Buffer 4 (New England Biolabs), 1× BSA and 10 U of *Hha*1 and added to 15 µl of pooled PCR product. Samples were incubated at 37°C for 5 h and were subsequently heat inactivated for 20 min at 65°C. The digested DNA was then precipitated from the solution by adding 2 µl of NaOAc (pH 5.2) and 50 µl of 95% ice-cold ethanol and centrifuged at 13 000 × *g* for 5 min at 4°C. The ethanol was removed, and the DNA was again washed with 100 µl of 70% ethanol. All ethanol was removed from the sample and air dried until residual alcohol had evaporated. The DNA pellets were re-suspended in Beckman Coulter solutions (Beckman Coulter Inc.) containing 0.25 µl of size standard (600 bp) and 39.75 µl of SLS and were loaded onto a 96-well sample plate. Plates were centrifuged briefly at 500 × *g*, and each digest was then overlaid with 1 drop of mineral oil. Digested samples were separated on a Beckman Coulter CEQ 8800 sequencer. Fragment analysis was performed on an 8 capillary array in fragment analysis mode using Beckman Coulter CEQ 8000 software. The parameters included a modified Fragment 4 injection time of 20 s, with a ramp to 2 kV over 2 min and run at 4.9 kV and 60°C for 60 min. Peak size and retention times were exported into T-align (Smith et al. 2006), and consensus peaks were determined by aligning replicate peak profiles and including any peaks within a range of 0.5 peak area. Peak profiles were then converted into a presence/absence matrix and visualized using nMDS with Euclidean distances.

Sequence phylogenetic analysis and diversity indices. Sequences from clone libraries were checked for chimera formation with the CHECK_CHIMERA software of the Ribosomal Database Project (Maidak et al. 1996). Sequence data (approximately 700 bp each) were aligned to the closest relative using the BLAST database algorithm (Altschul et al. 1997), and all sequence affiliations were determined by >97% identity to bacterial 16S rRNA gene sequences in the GenBank database. Sequence affiliations were cross-validated with the Greengenes database (http://greengenes.lbl.gov) (DeSantis et al. 2006) using the Hugenholtz and Ribosomal Database Project (RDP) (Maidak et al. 1996) alignments, and sequences that closely affiliated with uncultured bacteria were assigned taxonomic affiliations using RDP database alignments. Sequences were assigned operational taxonomic units (OTUs) by comparing sequences with the same affiliations and grouping those with >97% similarity. The OTUs of library clone and DGGE sequences were imported into the ARB software package (www.arb-home.de) (Ludwig et al. 2004), aligned against the Greengenes database (http://greengenes.lbl.gov) (DeSantis et al. 2006) that is compatible with ARB and subject to manual correction of the alignment when necessary. OTUs that were >2% of any juvenile clone library were included in a phylogenetic tree with dominant bacterial sequences retrieved from adult samples (Littman et al. 2009) to determine which conserved adult bacteria are present in juvenile samples. DGGE sequences were also included in the tree to determine sequence affiliations as well as crossvalidate sequence alignments with clone library data. Tree topologies were evaluated by reconstructing phylogenies using evolutionary distance (Phylip distance method with the Jukes and Cantor model) analysis of aligned near full-length sequences (>1000 bp) (Ludwig et al. 1998). Ambiguous

sequence regions were removed from the analysis. Aligned, partial 16S rRNA gene sequences (<1000 bp) were subsequently inserted without changing the overall tree topology using the parsimony tool available within ARB. The nucleotide sequence data of clones and DGGE sequences appear in the GenBank nucleotide database under the Accession Numbers GQ301209 to GQ301527.

Diversity indices were compared for adult and juvenile *Acropora millepora* and *A. tenuis* containing the same *Symbiodinium* clades. The Shannon-Weaver diversity index (Shannon & Weaver 1963), the Chao1 richness estimator (Chao 1987) and Fisher's Alpha log series richness index (Fisher et al. 1943) were calculated using the freeware program EstimateS Win 7.52 (Colwell 2006). Coverage values were calculated by the equation: $C = 1 - (n/N) \times 100$, where n is the number of unique clones and N is the total number of clones examined in the libraries (Good 1953). The diversity of clone libraries was further investigated by rarefaction analysis (Hurlbert 1971, Heck et al. 1975, Simberloff 1978). Rarefaction curves were produced by using the analytical approximation algorithm of Hurlbert (1971). Calculations were performed with the freeware program aRarefact Win (Holland 1988).

RESULTS

Bacterial community profiles of 9 mo old juveniles

A total of 81 *Acropora tenuis* juveniles from the *Symbiodinium* D treatment and 126 from the *Symbiodinium* C1 treatment were alive when surveyed after 9 mo (September 2007). On average, D juveniles contained 23 ± 4 polyps and were (0.5 to 1 cm in diameter), in contrast to C1 juveniles, which contained approximately 51 ± 9 polyps and were visibly larger (1 to 2 cm). For *A. millepora*, 63 juveniles from the D treatment and 123 juveniles from the C1 treatment were alive at the time of the September sample. Average polyp counts (D juveniles: 18 ± 5; C1 juveniles: 50 ± 8) and colony sizes (D juveniles: 0.5 to 1.0 cm; C1 juveniles: 1 to 2 cm) of the *A. millepora* juveniles were similar to those of *A. tenuis* juveniles from the respective *Symbiodinium* C1 and D treatments (Table 1).

Bacterial communities identified within 16S rRNA gene clone libraries of all 9 mo old coral juveniles were dominated by α- and γ-*Proteobacteria*-affiliated sequences. No differences in dominant phylogenetic groups at the class level were discernable among corals containing different *Symbiodinium* clades (Table S1, available in the electronic supplement at www.int-res.com/articles/suppl/m389p045_app.pdf). Retrieved sequence diversity within the libraries examined at the genus and family levels demonstrated high bacterial diversity, and there were few similarities between coral juveniles, either when compared between *Acropora tenuis* and *A. millepora* juveniles associated with the same *Symbiodinium* type, or when compared between C1 and D juveniles within a species. Each juvenile clone library correlated with different dominant bacteria genera; there were also few similarities between any of the bacterial communities associated with the replicate juveniles within each coral species (Table 2). However, a few bacterial sequence OTUs appeared within replicate libraries more frequently, suggesting that juvenile libraries may have limited structure. The sequences that were recovered from multiple libraries, from both *A. millepora* and *A. tenuis* libraries hosting both *Symbiodinium* Clades C1 and D, include OTU-007 (affiliated with *Flavobacteria* sp.), OTU-009 (related to *Muricauda* sp.), OTU-168 (related to *Roseobacter* sp.), OTU-290 (related to *Achromobacter* sp.), OTU-308 (related to *Pseudomonas* sp.) and OTU-310 (affiliated to *Serratia* sp.). Two OTUs (148 and 149) related to *Brevundimonas* sp. appeared within most libraries, while a *Stenotrophomonas* sp.–related sequence (OTU-314) represented a large component of each library (between 2.2 and 31.7% of clones). Nevertheless, most common sequences listed above do not appear in every library and vary drastically in their relative proportions (Table 2). In addition, all juvenile clone libraries possessed a large number of independent bacterial ribotypes that only appeared once in each library, which is represented by the large number of OTU groups identified (Table S1). Due to the high variability and diversity within all juvenile libraries, no consistent patterns could be detected between samples harbouring Clade C1 or D *Symbiodinium* by directly comparing relative abundances of retrieved 16S rRNA gene sequences.

Bacterial community profiles assessed by DGGE displayed variable and complex banding patterns for replicate samples of both coral species. Sequences recovered from replicate DGGE sample profiles affiliated with *Brevundimonas* sp., *Stenotrophomonas* sp., *Muricauda* sp., *Achromobacter* sp. and *Ruegeria* sp. and corresponded to sequences retrieved from many *Acropora tenuis* and *A. millepora* clone library sequences (Table 2). This confirms that some bacterial ribotypes were consistent despite high diversity and variability in bacterial profiles. The nMDS plots representative of DGGE profiles for colonies of both *A. tenuis* (Fig. 1A) and *A. millepora* (Fig. 1B) show limited grouping of samples containing each of the 2 *Symbiodinium* clades, indicating that, as suggested by the clone library data, there was no obvious relationship between the clade of *Symbiodinium* harboured and the bacterial community composition in

juveniles of these 2 corals. Bacterial diversity fingerprints generated from tRFLP peak patterns were consistent with both clone library and DGGE analysis in showing no strong grouping or consistency in peak patterns for replicate juvenile samples (Fig. 1C,D). While nMDS representation of tRFLP peak patterns displayed some grouping of *A. tenuis* juveniles containing Clade D *Symbiodinium* (Fig. 1C), other replicate samples were dispersed. C1 juveniles, in particular, displayed little consistency in this species. *A. millepora* juveniles (Fig. 1D) differed between replicate samples possessing the same clade, for those associated both with *Symbiodinium* C1 and D (Fig. 1D), again indicating no clear relationship between the *Symbiodinium* type harboured and the bacterial community composition.

Table 2. Proportions of dominant operational taxonomic units (OTU) retrieved from 16S rRNA gene juvenile clone libraries (%). Sequences included comprise >2% of any juvenile clone library; sequences were aligned to the closest relative using BLAST (Altschul et al. 1997). The similarity was calculated without taking gaps into account. MC: *A. millepora* hosting Clade C1; MD: *A. millepora* hosting Clade D; TC: *A. tenuis* hosting Clade C1; TD: *A. tenuis* hosting Clade D; S-TC: *A. tenuis* hosting Clade C1 sampled in summer; S-TD: *A. tenuis* hosting Clade C1 sampled in summer; Unc.: unclassified

Clone	Closest relative	Identity	Affiliation	MC1	MC2	MD1	MD2	TC1	TC2	TD1	TD2	S-TC1	S-TC2	S-TD1	S-TD2
OTU-001	*Acidobacteria* (DQ289940)	98	*Acidobacteria*						2.4	1.2					
OTU-004	*Bacteroidetes* bacterium (AY162097)	99	*Bacteroidetes*			1.2	1.1	2.4	3.5						
OTU-007	*Flavobacteria* (AF277542)	98	*Bacteroidetes*	4.3		1.2	2.3	1.2	1.2	1.2		8.3	5.3		
OTU-009	*Muricauda* sp. (AY576744)	99	*Bacteroidetes*	2.2		1.2		2.4				2.1	2.6		
OTU-015	*Lyngbya* sp. (AB045906)	93	*Cyanobacteria*	3.2											
OTU-016	*Oscillatoria* sp. (AB058224)	96	*Cyanobacteria*	1.1		2.4	2.3								
OTU-020	*Synechococcus* sp. (AF132772)	91	*Cyanobacteria*	4.3											
OTU-023	Unc. bacterium (AF424415)	95	*Firmicutes*	1.1		2.4									
OTU-024	Unc. bacterium (AY258098)	97	α-*Proteobacteria*	1.1		2.4									
OTU-058	Unc. bacterium (AY942776)	97	α-*Proteobacteria*			2.4	5.7								
OTU-064	Unc. bacterium (AY568808)	95	δ-*Proteobacteria*				4.5								
OTU-011	Unc. bacterium (EF378470)	97	*Bacteroidetes*					1.2				6.3	1.3		
OTU-144	*Agrobacterium* sp. (AB247617)	99	α-*Proteobacteria*	2.2			1.1			1.2					
OTU-148	*Brevundimonas* sp. (AB426561)	99	α-*Proteobacteria*				1.1	13.4	1.2	25.9	21.1	10.4	2.6	1.4	3.1
OTU149	*Caulobacteraceae* (DQ857204)	99	α-*Proteobacteria*	2.2	2.3				4.7		7.8				
OTU-150	*Erythrobacter* sp. (DQ985055)	98	α-*Proteobacteria*		4.7	2.4									3.1
OTU-153	*Kordiimonas* sp. (AY682384)	93	α-*Proteobacteria*	1.1					2.4						
OTU-155	*Mesorhizobium* sp. (DQ917826)	98	α-*Proteobacteria*				3.4								6.3
OTU-161	*Rhodobacteraceae* (AY962292)	99	α-*Proteobacteria*	4.3					1.2						
OTU-165	*Rhodopseudomonas* sp. (AY428572)	93	α-*Proteobacteria*				4.5								
OTU-168	*Roseobacter* sp. (AY745856)	99	α-*Proteobacteria*					1.2	1.2	3.5	3.3		5.3	4.2	
OTU-170	*Ruegeria* sp. (AJ391197)	99	α-*Proteobacteria*				3.4		1.2					6.9	
OTU-171	*Silicibacter* sp. (AY369990)	99	α-*Proteobacteria*	1.1			3.4							1.4	
OTU-173	*Sphingomonas* sp. (EU817494)	99	α-*Proteobacteria*									8.3	2.6		3.1
OTU-177	Unc. α-*Proteobacterium* (AY499896)	95	α-*Proteobacteria*	6.5			1.1								
OTU-184	Unc. α-*Proteobacterium* (AJ890099)	99	α-*Proteobacteria*	3.2		1.2									
OTU-191	Unc. α-*Proteobacterium* (AJ633940)	99	α-*Proteobacteria*			7.2									
OTU-202	Unc. α-*Proteobacterium* (AM162572)	89	α-*Proteobacteria*				3.4								
OTU-218	Unc. α-*Proteobacterium* (DQ107390)	99	α-*Proteobacteria*					1.2	2.4						
OTU-262	Unc. α-*Proteobacterium* (DQ003179)	99	α-*Proteobacteria*							3.5					
OTU-279	Mucus bacterium (AY654746)	99	α-*Proteobacteria*	5.4		4.8									
OTU-290	*Achromobacter* sp. (EU275167)	100	β-*Proteobacteria*		7.0	1.2				8.2	2.2				
OTU-291	*Delftia* sp. (EU019989)	99	β-*Proteobacteria*							3.5					
OTU-294	Unc. β-*Proteobacterium* (EF061949)	99	β-*Proteobacteria*			3.6									
OTU-293	Unc. β-*Proteobacterium* (AB288554)	99	β-*Proteobacteria*	1.1						3.5					
OTU-299	*Alteromonas* sp. (EU529840)	100	γ-*Proteobacteria*	3.2								2.1		1.4	
OTU-300	*Escherichia* sp. (AM087500)	99	γ-*Proteobacteria*			1.2					1.1	4.2			3.1
OTU-308	*Pseudomonas* sp. (AY014801)	99	γ-*Proteobacteria*			3.6	3.4	1.2		1.2		2.1		6.9	
OTU-310	*Serratia* sp. (AY566180)	99	γ-*Proteobacteria*			1.2	3.4			1.2	2.2				
OTU-313	*Spongiobacter* sp. (AB205011)	98	γ-*Proteobacteria*			1.2		2.4		1.2	1.1				
OTU-314	*Stenotrophomonas* sp. (AM402950)	100	γ-*Proteobacteria*	2.2	27.9	8.4	4.5	31.7	8.2	29.4	24.4			2.8	
OTU-315	*Thalassomonas* sp. (AY194066)	98	γ-*Proteobacteria*	9.7			1.1		1.2						
OTU-348	*Vibrio* sp. (AJ316167)	99	γ-*Proteobacteria*											13.9	
OTU-349	Unc. α-*proteobacterium* (DQ146982)	99	γ-*Proteobacteria*	5.4			1.1				1.1				
OTU-350	*Vibrio* sp. (EU372927)	99	α-*Proteobacteria*												18.8
OTU-355	*Haliangium* sp. (AB062751)	97	δ-*Proteobacteria*			3.6									
OTU-356	*Myxobacterium* (AB016469)	97	δ-*Proteobacteria*			1.2			2.4						

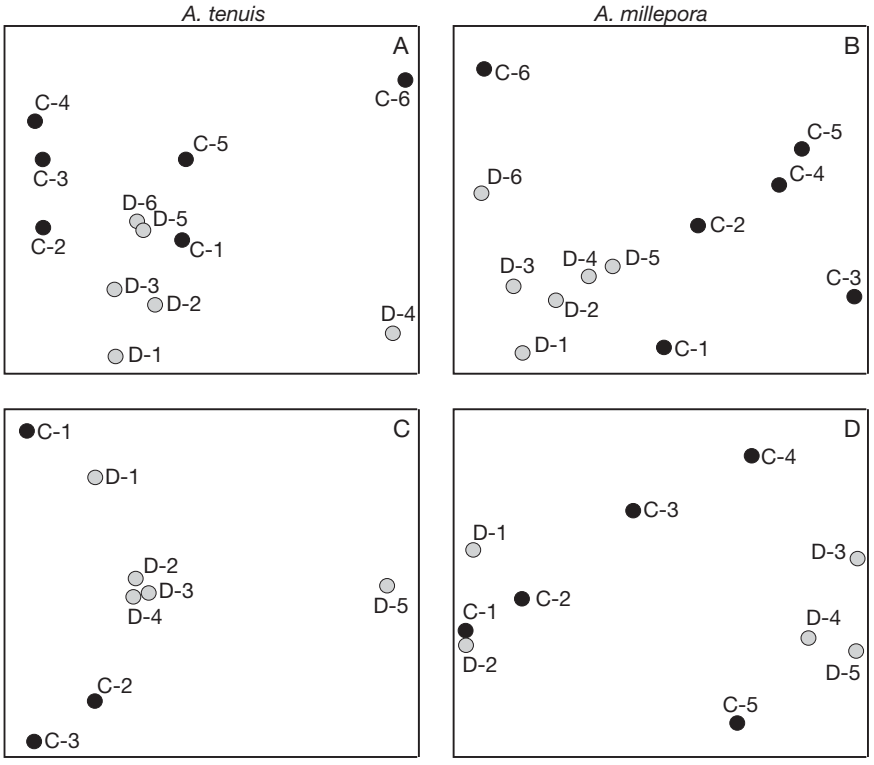

Fig. 1. *Acropora tenuis* and *A. millepora*. Comparison of 9 mo old juvenile corals containing Clade C1 and D *Symbiodinium* by non-metric multidimensional scaling (nMDS) of bacterial profiles. Denaturing gradient gel electrophoresis (DGGE) profiles of (A) *A. tenuis* and (B) *A. millepora*. Terminal restriction fragment length polymorphism (tRFLP) profiles of (C) *A. tenuis* and (D) *A. millepora*. Several replicate samples are missing in the tRFP analysis such as D-6, C-4, C-5 and C-6 for *A. tenuis* and C-6 for *A. millepora*, due to poor reads by the sequencer and resulting loss of DNA after repeated trials, which may contribute to the lack of pattern observed in the plot

9 mo old juvenile versus adult bacterial communities

Diversity parameters associated with clone libraries generated from the 9 mo old juvenile samples were compared with bacterial diversities of adult corals associated with the corresponding *Symbiodinium* clades and collected from the same sites (Littman et al. 2009). Only clone libraries of 9 mo old corals with the same *Symbiodinium* clade to those of the adult libraries were included in the analysis, such as *Acropora millepora* harbouring Clade D and *A. tenuis* hosting Clade C1, to eliminate symbiont type as a possible influence. A total of 141 OTUs (grouped at 97% sequence identity) were identified within *A. millepora* juvenile coral libraries compared to only 53 OTUs from libraries of adult corals (Table 3). For both juvenile and adult *A. millepora* samples, libraries were pooled for the analysis, resulting in a total of 187 clones included in each analysis. For *A. tenuis*, 107 OTUs were identified within pooled juvenile libraries compared to 49 OTUs for adult *A. tenuis* (out of 179 and 183 clones, respectively). Many sequences retrieved from juveniles affiliated with previously unclassified sequences and were the sole ribotypes within a defined OTU. Other bacterial ribotype richness (Chao1 and the Fisher abundance model) and evenness (Shannon-Weaver index) indices supported

Table 3. *Acropora millepora* and *A. tenuis*. Diversity indices calculated from operational taxonomic units (OTUs) (97% similarity) of 16S rRNA clone libraries. Clones: no. of clones analysed; coverage: coverage of clone libraries; OTU: observed no. of OTUs; *H′*: Shannon-Weaver diversity index; Chao1: Chao's richness estimator; α: Fisher's alpha; *D*: Simpson's evenness

	Clones (n)	Coverage (%)	OTU (n)	*H′*	Chao1	α	*D*
A. millepora							
Adult	187	71.7	53	3.4	80.2	24.7	21.74
Juvenile	187	24.6	141	4.8	474.2	260.9	238.2
A. tenuis							
Adult	183	73.2	49	3.3	72.2	21.9	19.7
Juvenile	179	43.0	102	4.2	280.3	98.4	50.1

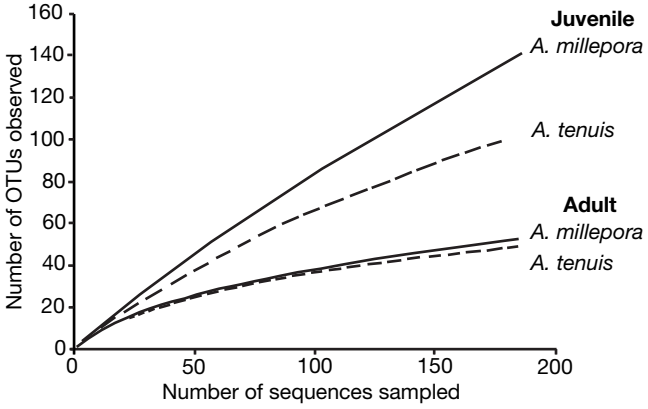

Fig. 2. *Acropora millepora* and *A. tenuis*. Rarefaction analysis of adult and 9 mo old juvenile *A. millepora* and *A. tenuis*. *A. millepora* corals compared harbour Clade D, while *A. tenuis* corals contain Clade C1 *Symbiodinium*. OTUs: operational taxonomic units

the conclusion that diversity was higher within juvenile samples (Table 3). The obtained sequences covered a high percentage of the diversity in the adult libraries (72 and 73% for *A. millepora* and *A. tenuis* libraries, respectively) in comparison to juvenile libraries (25 and 43% for *A. millepora* and *A. tenuis* libraries, respectively). Rarefaction curves confirmed the coverage calculations, with juvenile libraries failing to reach an asymptote in comparison to curves for adult corals (Fig. 2). Although greater numbers of sampled clones would better represent bacterial diversity for all libraries, it was apparent that bacterial diversity of juvenile corals was vastly undersampled compared to that of adult corals.

nMDS representation and direct comparisons of juvenile and adult coral bacterial DGGE profiles displayed tight grouping of bacterial fingerprints for adult corals of both *Acropora tenuis* (Fig. 3A) and *A. millepora* (Fig. 3B). Noticeably, the juvenile samples were widely spaced within the nMDS representation of DGGE fingerprints, indicating high variability in profiles in comparison to adult profiles. Similarly, nMDS plots of tRFLP profiles showed much tighter grouping of adult coral samples relative to juvenile sample profiles for both species (Fig. 3C,D).

Most sequences from adult *Acropora millepora* and *A. tenuis* clones libraries were closely related to sequences retrieved from juvenile libraries. For example, phylogenetic analysis of dominant OTUs from juvenile libraries including OTUs 148 and 149 related to *Brevundimonas* sp., 313 related to *Spongiobacter* sp., 314 related to *Stenotrophomonas* sp., 168 related to *Roseobacter* sp.

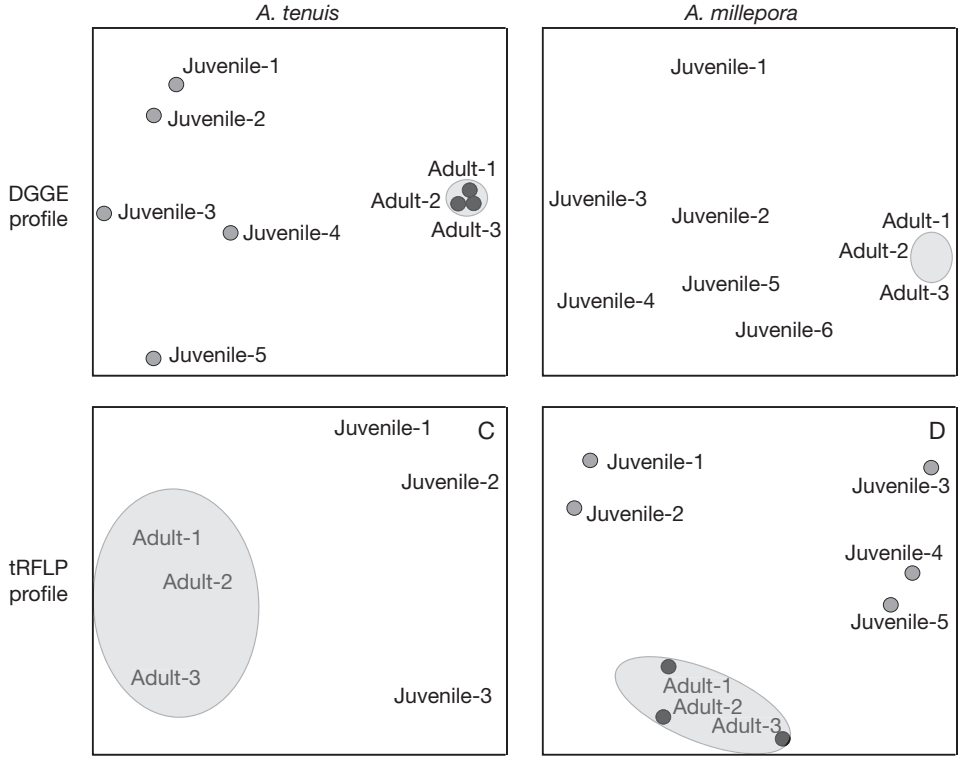

Fig. 3. *Acropora millepora* and *A. tenuis*. Comparison of 9 mo old juvenile and adult corals by nMDS of bacterial profiles. DGGE profiles of (A) *A. tenuis* and (B) *A. millepora*. Terminal restriction fragment length polymorphism (tRFLP) profiles of (C) *A. tenuis* and (D) *A. millepora*

and 161 of the *Rhodobacter* group all correspond to dominant sequences retrieved from adult clone libraries (Fig. S1, available at www.int-res.com/articles/suppl/m389p045_app.pdf). Therefore, despite the high diversity and variability within juvenile libraries, many of the adult sequences are present in varying relative proportions, although not consistently detected in all libraries (Table 2). Also, a few dominant sequences found on adult corals were not retrieved from juvenile samples, including the δ*Proteobacterium* (FJ489722), *Anaeromyxobacteria*-related sequence (FJFJ489711) and *Marinobacter*-related sequence (FJ489730).

Temporal changes in bacterial communities of *Acropora tenuis*

Survival of juvenile colonies of *A. tenuis* was reduced after 12 mo (December 2007; Austral summer) when water temperatures reached 30°C. At this sampling date, only 18 individuals (22%) from the *Symbiodinium* D treatment remained alive, compared to 81 colonies when sampled 3 mo earlier. Colonies from the *Symbiodinium* D treatment exhibited little growth, and average colony size remained at approximately 20 polyps (about 1 cm in diameter). In contrast, survival of *A. tenuis* from the *Symbiodinium* C1 treatment was 4-fold greater (112 colonies or 89% remained alive) and colonies were noticeably larger in size (approximately 2 to 3 cm) (Table 1).

Clone libraries of 1 yr old *Acropora tenuis* juveniles infected with different clades of *Symbiodinium* displayed similar proportions of bacterial classes, although a greater proportion of γ-*Proteobacteria* affiliated sequences were recovered from *A. tenuis* hosting Clade D (33 and 34% of the sequences, respectively) in comparison to libraries of *A. tenuis* hosting Clade C1 (11 and 17%, respectively). Bacterial profiles derived from clone libraries were analysed at the genus and family levels using PCA. The dominant ribotypes (grouped at 97% sequence identity) included in the PCA comprised >2% of any library compared and accounted for 88% of the variability (Fig. 4). The PCA results for 1 yr old *A. tenuis* revealed 1 distinct difference between samples hosting Clade D and C1 *Symbiodinium*. For both *A. tenuis* libraries contain-

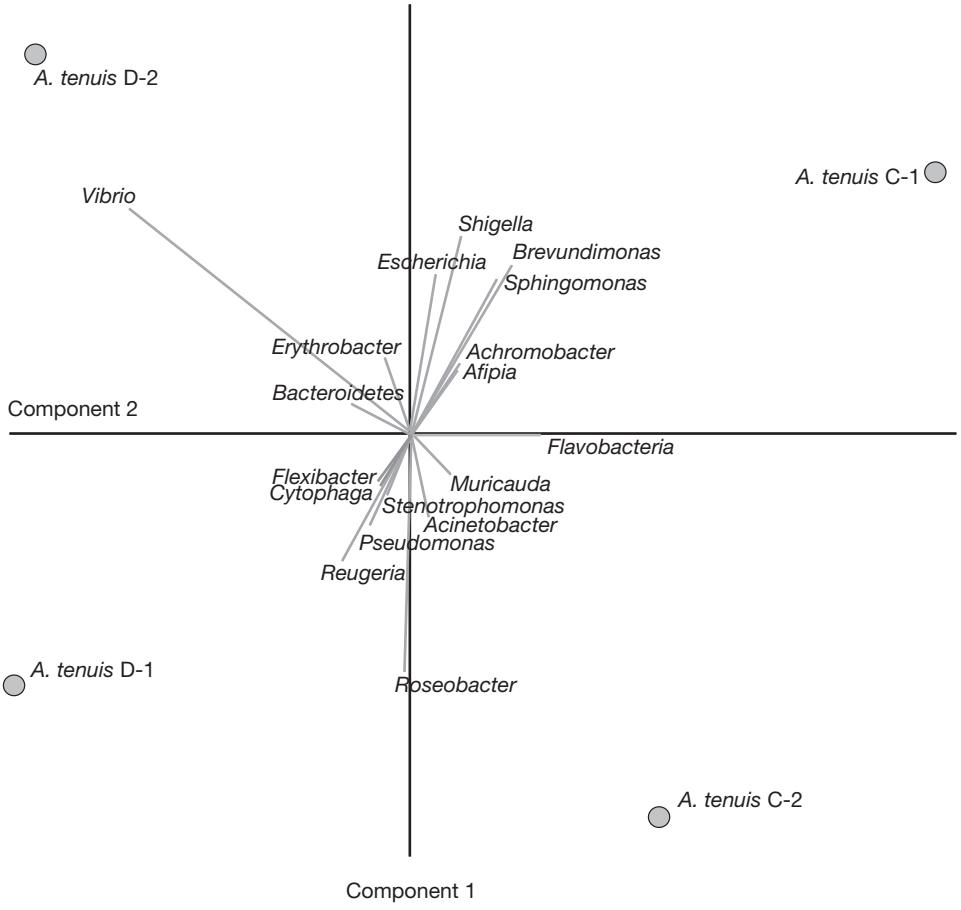

Fig. 4. *Acropora tenuis*. Principal components analysis of 1 yr old juveniles harbouring Clade C1 and D *Symbiodinium*

ing Clade D, the sequences OTU-348 and -350, affiliating with *Vibrio* sp., were the dominant bacterial ribotype retrieved and were displayed as a prominent vector on the PCA plot. However, less dominant sequences within the libraries separated the 2 *A. tenuis* Clade D libraries on the PCA plot. The sequences retrieved from *A. tenuis* Clade D Library 1 affiliated with *Vibrio* sp. (13.9% of the library), *Pseudomonas* sp. (6.9% of the library), *Ruegeria* sp. (6.9% of the library) and *Roseobacter* sp. (4.2% of the library), while the dominant sequences retrieved from *A. tenuis* Clade D Library 2 affiliated with *Vibrio* sp. (18.8% of the library) and other *Bacteroidetes* (6.3% of the library) (Table 2). In contrast, no sequences affiliated with *Vibrio* sp. were retrieved from the *A. tenuis* libraries that contained Clade C1 *Symbiodinium*. Variation within the dominant retrieved sequences of Clade C1 corals also caused separation of libraries on the PCA plot. Dominant sequences within the *A. tenuis* Clade C1 library affiliated with *Brevundimonas* sp. (10.4% of the clone library), *Flavobacteria* sp. (8.3% of the library), *Shigella* sp. (6.3% of the library) and *Sphingomonas* sp. (8.3% of the library), whereas the sequences that were most prevalent in the *A. tenuis* Clade C2 library affiliated with *Flavobacteria* (5.3% of the library) and *Roseobacter* sp. (5.3% of the library) (Table 2). The *Vibrio* sp. affiliated sequences within the Clade D *A. tenuis* libraries accounted for the 2- to 3-fold increase in the proportion of γ-*Proteobacteria*-affiliated clones within these libraries compared to the Clade D *A. tenuis* libraries. The *Vibrio*-like sequences OTU-348 and -350 were closely related (99% identity) to the known coral pathogens *Vibrio coralliilyticus* (AJ316167) and *Vibrio* sp. (AJ316170) isolated from white syndrome (Fig. S1).

Distinctly different DGGE bacterial banding patterns were observed for replicate 1 yr old samples of *Acropora tenuis* corals infected with D versus C1 *Symbiodinium* (Fig. 5A). nMDS representation of these DGGE profiles demonstrated grouping of Clade D corals (Fig. 5B). The dominant band (Fig. 5A; Band K) within Clade D profiles was related to *Vibrio* species, including a *Vibrio* sp. (98% sequence identity) isolated from white syndrome (EU372927) (Table 4, Fig. S1), confirming observations that *Vibrio* spp. comprised a large component of the 1 yr old juvenile clone libraries harbouring Clade D. Interestingly the 1 yr old juveniles hosting Clade C1 also appeared to have profiles that were consistent between replicate samples, with Bands A to J (Table 4) present in all profiles (Fig. 5A). This was in contrast to the profiles of 9 mo old juveniles that lacked consistent dominant microbial associations.

DISCUSSION

Juvenile corals lack conserved microbial associates

Coral microbial investigations over the last decade have highlighted the important role that the host's microbial partners, including photosynthetic *Symbiodinium* (zooxanthellae) and associated bacterial, fungal and archaeal microbiota, play in coral health (Knowlton & Rohwer 2003). These studies have suggested that some adult corals conserve their bacterial partners, based on evidence that these corals harbour species-specific bacterial communities (Rohwer et al. 2001, 2002). More recently, it has been shown that, in some coral species, bacterial associations vary between reef locations (Littman et al. 2009), and the current study demonstrates that juvenile hosts do not establish the conserved microbial patterns characteristic of adult corals at early development stages. Support for this latter conclusion was based on the absence of

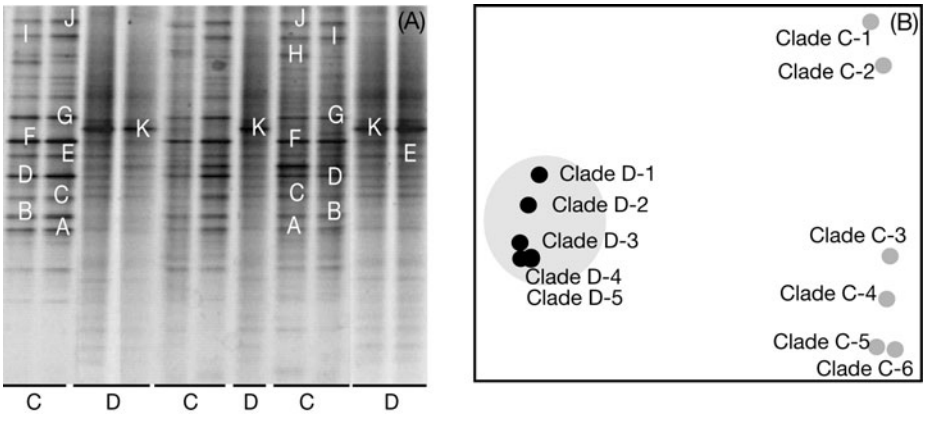

Fig. 5. *Acropora tenuis*. DGGE profiles for 1 yr old *A. tenuis* harbouring Clade C1 and D *Symbiodinium*. (A) DGGE gel of samples containing different clades. Bands labelled A to K. (B) nMDS plot of DGGE profiles

Table 4. *Acropora tenuis*. Affiliation of DGGE bacterial sequences retrieved from 1 yr old juvenile coral colonies (n = 11). Sequences were aligned to the closest relative using BLAST (Altschul et al. 1997). The similarity was calculated without taking gaps into account

Band	Closest relative and database accession number	Alignment (bp)	Similarity (%)	Taxonomic description
A	Uncultured bacterium (DQ684483)	241/304	79	γ-*Proteobacteria*[a]
B	Uncultured bacterium (EU808101)	292/294	99	α-*Proteobacteria*[a]
C	*Sphingobium* sp. (EU679660)	270/295	91	α-*Proteobacteria*
D	Uncultured bacterium (EU172379)	300/303	99	β-*Proteobacteria*[a]
E	Uncultured bacterium (EU535510)	270/309	87	β-*Proteobacteria*[a]
F	*Achromobacter xylosoxidans* (EU877076)	274/285	96	β-*Proteobacteria*
G	*Alcaligenes* sp. (EU304282)	300/303	99	β-*Proteobacteria*
H	Uncultured bacterium (EU010191)	287/315	91	
I	*Flavobacterium frigidarium* (AY771738)	304/309	98	*Bacteroidetes*
J	*Flavobacterium frigidarium* (AY771738)	298/308	96	*Bacteroidetes*
K	*Vibrio* sp. (EU372935)	307/311	98	γ-*Proteobacteria*

[a]Taxonomic description determined by phylogenetic analysis (Fig. S1, available at www.int-res.com/articles/suppl/m389p045_app.pdf)

conserved patterns in bacterial communities using bacterial 16S rRNA gene profiles from colonies of 9 mo old *Acropora tenuis* and *A. millepora* and 3 independent community profiling methods (clone libraries, DGGE and tRFLP analyses). Comparisons with a previous study of adults of the same coral species associated with the same *Symbiodinium* clades (Littman et al. 2009) highlight differences between juvenile and adult profiles, notably the highly conserved microbial profiles observed in adult corals using all three 16S rRNA gene profiling methods. Clone libraries of adults displayed lower relative diversity compared to juveniles, as determined by both diversity indices and rarefaction analysis, while nMDS interpretations of DGGE and t RFLP profiles displayed tight grouping of adult coral profiles in contrast to no apparent relationship among juvenile coral profiles.

Results from the present study also indicate that juvenile corals at 9 mo of age do not show any discernable relationship between the *Symbiodinium* clade present in the coral host and the bacterial community structure. Previous research has demonstrated that genetically different *Symbiodinium* clades differentially affect host physiology, resulting in, for example, different heat tolerances or growth rates (Fitt 1985, Baker et al. 2004, Little et al. 2004, Berkelmans & van Oppen 2006, Abrego et al. 2008). Since *Symbiodinium* partners exude substantial amounts of assimilated carbon into the mucus layer (Ikeda & Miyachi 1995), variation in *Symbiodinium* clades may affect the composition of coral mucus and, subsequently, select variant coral-associated microbiota, partly explaining the species-specific associations in previous studies (Littman et al. 2009). However, clone libraries, DGGE and tRFLP analyses all consistently showed no conserved diversity or specific associations for Clade C1 versus D infected *Acropora tenuis* and *A. millepora* colonies at 9 mo. We therefore conclude that the coral's *Symbiodinium* clade is not a principal factor driving differences in microbial partners in early developmental stages of these 2 coral species and that, at this early developmental stage, the high bacterial diversity found compared to that of adults indicates that no selection of bacterial partners is taking place. A lack of specificity for microbial associations in early ontogeny has also been observed sin the initial uptake of different *Symbiodinium* types. For instance, the apparent specificity for Strain C1 observed in adult populations of *A. tenuis* is not present in the early stages of infection (Little et al. 2004). New recruits take up both C1 and D strains and become dominated by *Symbiodinium* Clade D after ~4 mo, although Type C1 eventually dominates, either through competition between different symbiont types or host mediated up-regulation (Little et al. 2004). It is noteworthy that the establishment of *Symbiodinium* partners in early ontogeny is a dynamic process, and it can take up to 3 yr to establish adult patterns of *Symbiodinium*–coral symbioses (Abrego et al. 2009).

It has been proposed that a lack of specificity for symbionts may serve as an adaptive mechanism for establishing associations with multiple symbionts that have different physiological characteristics (Little et al. 2004, Abrego et al. 2009). Similarly, multiple bacterial types may settle on new corals until coral–bacterial interactions lead to an established community best suited for particular environmental conditions. This may explain why certain ribotypes appear with different levels of dominance within the juvenile profiles, such as *Stenotrophomonas*- and *Brevundimonas*-related sequences, observed frequently in DGGE profiles and in higher proportions in some clone libraries. Furthermore, phylogenetic analysis of dominant clone

sequences from both adults and juveniles showed some similarities. Many sequences retrieved from adult corals were also found in the 9 mo old juvenile libraries (Fig. S1), suggesting that the juveniles may be in the process of establishing their adult associations. However, many sequences retrieved from adult corals were not detected in juvenile clone libraries, possibly suggesting a successional process whereby the diverse bacterial communities are gradually replaced by the adult associates. Such a winnowing process (Nyholm & McFall-Ngai 2004) is similar to patterns emerging for the establishment of *Symbiodinium* endosymbiosis (Rodriguez-Lanetty et al. 2006) and may reduce the comparatively high bacterial diversity of juveniles and result in the establishment of conserved adult bacterial communities. Apprill et al. (2009) similarly found distinct communities of bacteria associated with oocyte bundles, spawned eggs and planula larvae of *Pocillopora meandrina*, supporting the observation that bacterial associates change throughout early developmental stages. However, planula larvae only internalized 1 *Roseobacter* clade after 79 h, indicating that coral can have specificity for associating with certain bacteria early in development. Future studies are required to examine the dynamics of bacterial associations throughout the full ontogeny of corals from larval stages through adulthood, to monitor the progression and establishment of a stable species-specific bacterial community.

Temperature stress on bacterial associations of juvenile coral

The greater mortality rates of *Acropora tenuis* juveniles hosting Clade D *Symbiodinium* (78%) in comparison to C1 juveniles (22% mortality), plus their 3-fold smaller sizes (1 cm diameter for D juveniles versus 3 cm diameter for C1 juveniles) (Table 1) indicate that *A. tenuis* juveniles harbouring Clade D were less fit and potentially received fewer nutrients for growth. Growth rates observed in the present study are supported by those recorded by Little et al. (2004), which showed a 2- to 3-fold higher growth rate in *A. tenuis* and *A. millepora* harbouring Clade C1 compared to Clade D. Clone libraries derived from *A. tenuis* juveniles harbouring Clade D possessed, in contrast to Clade C1 juveniles, a higher proportion of clones aligned with γ-*Proteobacteria*, with sequences related to *Vibrio* species constituting the majority of these clones. The DGGE bacterial profiles similarly demonstrated the prominence of *Vibrio* sp.–related sequences in *A. tenuis* samples hosting Clade D juveniles that were not apparent in Clade C1 juveniles. The appearance of *Vibrio*-like sequences as dominant members of the microbial communities in the seemingly unhealthy Clade D samples is noteworthy given that these organisms are often regarded as opportunistic pathogens in marine systems (Saeed 1995, Li et al. 1999, Kraxberger-Beatty et al. 2006), with some *Vibrio* species implicated specifically as coral pathogens (Kushmaro et al. 1997, Ben-Haim & Rosenberg 2002, Ben-Haim et al. 2003, Sussman et al. 2008). Moreover, *Vibrio*-like sequences retrieved from 1 yr old Clade D samples closely aligned with *Vibrio coralliilyticus* (Fig. S1), which has been demonstrated to be a temperature-dependent pathogen, causing tissue lysis of *Pocillopora damicornis* (BenHaim & Rosenberg 2002, Ben-Haim et al. 2003). Seawater temperatures were between 29 and 30°C at the time of sampling, indicating that the corals may have been experiencing thermal stress, increasing their susceptibility to pathogenic infection. For example, it has been demonstrated that *Vibrio shiloi* can switch on temperature-regulated virulence factors at 28°C, enabling bacterial infection and coral bleaching (Kushmaro et al. 1997, Ben-Haim et al. 1999, Banin et al. 2001). One study examined bacterial communities associated with corals through a bleaching event and demonstrated a relative increase in *Vibrio* sp.–related sequences prior to and during the peak stress of the bleaching period (Bourne et al. 2008). However, *Vibrio* spp. have also been shown to be normal constituents of the bacterial community associated with healthy coral (Littman et al. 2009), and the nitrogen-fixing capabilities of coral-associated *Vibrio* spp. may be important for the nitrogen cycling within the holobiont (Olson et al. 2009). Therefore, it is likely that *Vibrio* spp. normally reside on the surface of the coral, with environmental stressors, such as higher seawater temperatures, changing the homeostasis of coral microbial associates and permitting proliferation of these organisms. Nevertheless, the deterioration of coral juvenile health and correlation with *Vibrio* spp. dominance in clone libraries, as well as their frequency in DGGE bacterial profiles, imply that these organisms potentially play an important role in coral health. Further controlled temperature-stress experiments utilizing corals harbouring different *Symbiodinium* clades are required to establish whether the entire holobiont is more stable with certain types of *Symbiodinium* and how *Vibrio* spp. proliferation affects overall coral health.

The decreased fitness of corals hosting Clade D observed in our study has greater implications for coral resilience to climate change. Previous studies have suggested a higher tolerance to heat for corals associated with Type D (Glynn et al. 2001, Toller et al. 2001, Baker et al. 2004, Fabricius et al. 2004, Rowan 2004, Berkelmans & van Oppen 2006). For example, Berkelmans & van Oppen (2006) demonstrated that *Acropora*

millepora shifted the dominant symbiont from Clade C2 to D after bleaching, with subsequent experiments showing that corals were more thermally tolerant when hosting Clade D. However, these studies involved species in which Type D is homologous. Abrego et al. (2009) showed that *A. tenuis*, in which Type C1 is homologous, were more thermally tolerant with Clade C1 than with Clade D, suggesting that host factors are also involved in determining heat tolerance. Our results are consistent with this conclusion, indicating the Clade D *A. tenuis* may be more susceptible to bacterial proliferation under heat stress. Two studies have speculated that the *Symbiodinium* type may contribute to coral resistance to disease, providing possible explanations for differences in coral communities hosting different clades. Sussman et al. (2009) showed differential susceptibility of *Symbiodinium* clades to a metalloprotease, suggesting that coral (specifically *A. millepora*) may be more susceptible to photosystem II inactivation when hosting Clade A. However, corals harbouring Clade C1 and D did not show differences in this regard. Stat et al. (2008) found sub-optimal health states of *A. cytherea* harboring Clade A, as well as an increased incidence of disease, compared with coral harbouring Clade C. In addition, they demonstrated significantly higher amounts of carbon released by Clade C than Clade A, providing evidence for less nutrients passed on to the host and therefore decreased fitness of the host as a mechanism for disease susceptibility. The smaller sizes of juveniles hosting Clade D indicate less nutrient acquisition than those hosting Clade C1, suggesting a trade-off for corals when hosting Clade D versus C1. Further investigations are required to find possible consequences of corals shifting *Symbiodinium* type.

CONCLUSIONS

We conclude that colony age, heat stress and, potentially, the *Symbiodinium* type may contribute to the establishment and dynamics of bacterial communities associated with the coral holobiont. High and variable bacterial diversity on juvenile corals, as indicated by clone libraries, DGGE and tRFLP analysis, suggest that a winnowing process takes place throughout early developmental stages of juvenile coral growth to establish adult patterns of species-specific bacterial associations. Lower growth rates and higher mortality rates of D juveniles of *Acropora tenuis* indicate that they are less fit than C1 juveniles. Concomitant proliferation of *Vibrio* sp. on corals harbouring Clade D suggests that coral associated with *Symbiodinium* Type C1 may be less susceptible to opportunistic pathogens during times of environmental stress. Therefore, it is possible that the *Symbiodinium* clade may be important for supplying corals with enough energy to ensure a stable microbial community when subjected to elevated temperatures.

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