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Research Article

Widespread Oceanospirillaceae Bacteria in *Porites* spp.

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We present evidence that a clade of bacteria in the Oceanospirillaceae is widely distributed in *Porites* spp. and other hermatypic corals. *Bacteria* 16S rDNA clone libraries were prepared from community genomic DNA extracted from *Porites compressa* and *Porites lobata* surface mucus and adjacent seawater collected along a line transect off Maui. Phylogenetic affiliations of operational taxonomic units (OTUs) defined at the 97% level of nucleotide identity varied within and between the respective *Porites* spp. along the transect and differed from those in the seawater. One OTU (C7-A01), however, occurred in all mucus samples from both *Porites* species. C7-A01c affiliates with a clade of uncultivated *Oceanospirillum*-like bacteria; the nearest neighbors of this OTU have been reported only in the surface mucus layer of *Porites* spp. and other stony corals, in reef-dwelling invertebrates, and the corallivorous six-banded angelfish, *Pomacanthus sexstriatus*.

1. Introduction

The best known interaction between hermatypic corals and other organisms is that between *Symbiodinium* zooxanthellae and the coral host [1–3]. However, specific interactions may also exist between prokaryotes and hermatypic corals [4–10]. It is important to determine if such associations do exist, given how sensitive some corals are to environmental stressors and that microbes may be involved in a response [11]. In this context, the phylogenetic structure of the microbial flora in a healthy coral should be defined before predicting its role or function in diseased or otherwise stressed corals. Through 16S rDNA clone libraries, we investigated the phylogeny of microbial communities in mucus from apparently healthy *Porites compressa* and *P. lobata* corals along a line transect over a reef off the coast of Maui, Hawai'i. Through this sampling design, we aimed to determine if there is evidence that particular *Bacteria* species occur consistently in either or both *Porites* species and contemporaneously collected adjacent seawater and if any in turn affiliate phylogenetically with those reported in corals or other organisms elsewhere. Evidence for such associations should set the scene for cultivation attempts directed at these *Bacteria*, and, as with other animal-microbe interactions, elucidation of the mechanisms involved in establishing and maintaining the association [12].

2. Materials and Methods

2.1. Site Selection. A 150 m line-transect was established on the West Maui patch reef at 20° 48.399′N, 156° 36.064′W. Wave activity here is dampened by an outer barrier reef. Commercial and residential developments are absent. The annual rainfall of 42–579 mm on the leeward side of the West Maui Mountains delivers little terrestrial run-off and nutrients to this reef [13].

2.2. Sampling Design. Ten quadrats (numbered 1–10) were designated along a 150 m long line transect which began ∼100 m offshore of high water (quadrat 1), and extended roughly parallel to the shore to 30 m offshore at quadrat 10. Water was <3 m deep at all quadrats. Each quadrat was marked with flagging tape, placed to preclude damage to the corals. All quadrats were randomly selected, with the only criterion being that both healthy *P. lobata* and *P. compressa* colonies occurred in the 4 m^2 area. Such pairings thus determined where quadrats were located along the transect. Coral "health" was determined visually, with *P. lobata* colonies considered "healthy" and selected if they were consistently colored yellowish olive-green to brown, and *P. compressa* if they were yellow-to-grayish tan to light brown [14]. Growth anomalies, tissue swelling, white or

black banding, pockmarks or pink and pale swollen nodules, discoloration or color loss (bleaching), multifocal tissue loss, algal growth, and accumulated sedimentation were absent [15]. Massive colonies were sampled only if they fell entirely in the 4 m^2 quadrat.

2.3. Habitat Properties. Seawater salinity, dissolved oxygen concentration, turbidity, temperature, and pH were determined daily at each of the ten sampling blocks for four days prior to and on the day of *P. lobata* and *P. compressa* mucus collection. Dissolved oxygen concentrations and temperatures were determined *in situ* with an YSI EcoSense DO200 hand-held dissolved oxygen meter. Turbidity in water samples was determined on shore with a portable LaMotte 2020e nephelometer. Salinity and pH were also measured on shore, then confirmed upon return to the laboratory at the University of Hawai‘i. Ammonia, nitrate, nitrite, and phosphate concentrations in seawater were also determined at the University of Hawai‘i at Mānoa using Hach Nessler (method 8038), cadmium reduction (method 8039), diazotization (method 8507), and reactive phosphorus (method 8048) methods, respectively (Hach Company, Loveland, CO, USA).

2.4. Sample Collection. Mucus was taken from one colony each of *P. lobata* and *P. compressa* in all ten quadrats, along with seawater adjacent to each sampling quadrat, all on the fifth day. Coral mucus (125 mL per colony) was taken into separate sterile plastic 30 mL syringes each tipped with a 15 cm long sterile stainless steel cannula. Each cannula tip was placed gently on and moved along the coral surface as mucus was collected. Mucus-filled syringes were capped underwater and returned to the surface, where the mucus was immediately transferred to sterile 50 mL polypropylene tubes. Voucher samples prepared by adding 10 mL of mucus from each colony to sterile glycerol (20% w/v) were stored on ice for delivery to the Department of Microbiology at the University of Hawai‘i, whereupon they were stored at -20°C until community genomic DNA was extracted within seven days.

In each quadrat, seawater ~1 m from the point at which coral mucus was taken was collected into sterile 1 L Nalgene bottles that were opened and closed underwater. Water samples were returned to the surface and stored on ice during transfer to the University of Hawai‘i at Mānoa where they were stored at -20°C until extraction of community genomic DNA within seven days.

2.5. Genomic DNA Isolation. Coral mucus and seawater collected at five of the ten quadrats described above were selected for further analysis, using a random number generator to select the quadrat number. From each of the five randomly selected quadrats, unamended seawater samples were divided into two ~250 mL fractions and filtered through separate 0.22 μm pore size, 47 mm diameter S-PAK membrane filters (Millipore Corp., Billerica, MA, USA). Ten milliliters of each mucus sample were similarly filtered. All filters were immediately removed to a freezer (-20°C) until community DNA was extracted by (1) a modified phenol-chloroform extraction adapted from Reysenbach et al. [16] and (2) the MoBio UltraClean Soil DNA kit (MoBio Laboratories Inc., Carlsbad, CA, USA).

Phenol-chloroform extraction began with filter sections briefly vortexed in 2 mL of 10 mM sodium phosphate buffer (pH 7.0) with 20% sucrose (wt/v) and 2.5 mg mL^{-1} lysozyme (Fisher Scientific). After overnight incubation (37°C), 5 mL of lysis buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA, and 1% SDS) and 2.5 mg proteinase K mL^{-1} (Sigma Aldrich Co.) were added. After overnight incubation (37°C), the mixture was pipetted into 50 mL polypropylene centrifuge tubes. An equal volume of phenol : chloroform : isoamyl alcohol (25 : 24 : 1) (Fisher Scientific) was added to each tube and mixed by gentle inversion. After centrifugation (4068 ×g, 3 min), the aqueous top layer was pipetted into sterile 50 mL polypropylene centrifuge tubes and combined with an equal volume of chloroform : isoamyl alcohol (24 : 1) (Fisher Scientific). Tubes were centrifuged (4068 ×g, 3 min) after gentle mixing. The aqueous layer was transferred to sterile 1.5 mL microfuge tubes and combined with 3 M sodium acetate (pH 5.2) to a final concentration of 0.3 M and isopropanol (60% of the aqueous volume). Tubes were incubated (4°C) for 25 min, then centrifuged (16,060 ×g, 15 min, 4°C). The isopropanol was removed and DNA pellets washed with ice-cold 70% ethanol. After centrifugation (16,060 ×g, 3 min), the ethanol was removed and DNA pellets allowed to dry. DNA was then dissolved in 50 μL autoclaved, distilled, and deionized water, combined with DNA in other tubes from the same sample and added to the DNA precipitation step of the MoBio UltraClean Soil DNA kit; this kit was used to extract community genomic DNA from the second membrane filter of each sample, according to the manufacturer's instructions.

2.6. Gene Amplification. Polymerase chain reactions (PCRs) were comprised of *Bacteria*-specific primers (forward primer 8-27: 5′-AGAGTTTGATCCTGGCTCAG-3′ [17] and reverse primer 1492: 5′-GGTTACCTTGTTACGACTT-3′ [16]) at three annealing temperatures, 48, 52, and 55°C, for 35 cycles, with GoTaq DNA Polymerase (Promega Corporation, Madison, WI, USA). Amplified DNA was purified in the MoBio UltraClean PCR clean-up kit and pooled.

2.7. Construction of 16S rRNA Gene Clone Libraries. Cloning reactions used the TOPO TA cloning kit for Sequencing (Invitrogen Co., Carlsbad, CA, USA) and pCR 4-TOPO cloning vector. White colonies were selected using blue/white screening on SOB plates with 50 μg mL^{-1} kanamycin (Sigma Aldrich Co.) and 40 mg mL^{-1} 5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside (X-gal). 96 clones were transferred to 96-well plates containing 100 μL Luria-Bertani (LB) broth and 50 $\mu g \cdot mL^{-1}$ kanamycin per well. Plates were incubated overnight (37°C) in a Jencon Scientific Millennium plate shaker. Plasmid inserts in overnight cultures were amplified in PCRs with pCR 4-TOPO specific primers (M13 Forward (−20): 5′-GTAAAACGACGGCCAG-3′ and M13 Reverse: 5′-CAGGAAACAGCTATGAC-3′ [18]). Products were purified in MoBio UltraClean SpinBind solution and Eppendorf

DB 96-well vacuum filter plates (Eppendorf AG, Hamburg, Germany).

2.8. Sequencing. DNA inserts were sequenced in a core facility at the University of Hawai‘i at Mānoa. Only inserts of the correct size (∼1.4 kb) were sequenced; the number of inserts sequenced ranged from 88 to 189 for the five *P. compressa* samples, 84 to 187 for the five *P. lobata* samples, and 78 to 93 for the five seawater samples. More clones from the coral samples, especially *P. lobata*, were sequenced after rarefaction curves generated on the basis of a smaller number of inserts suggested that diversity was undersampled (data not shown). Consensus sequences were checked for chimeric features in Greengenes, the Ribosomal Database Project (RDP), and Bellerophon [19–21]. Nonchimeric sequences were compared with nucleotide sequences in the RDP, Greengenes, Community Cyberinfrastructure for Advanced Marine Microbial Ecology Research and Analysis (Camera), Silva, and GenBank [21] nonredundant databases using the gapped BLASTn search algorithm [22]. All such comparisons returned nearest neighbor and nearest type strain. Greengenes, RDP, Camera, and Silva returned gapped and nongapped alignments that were used as input in the ARB sequence analysis package. Rarefaction curves were generated in DOTUR (distance-based OTU richness) v1.53 [23] to estimate species accumulation based on ≤97% nucleotide identity criterion and to measure the number of OTUs detected as a function of effort. Shannon and Simpson diversity indices were generated in support of the richness estimates [23–25]. All nonredundant sequences from each sample were uploaded into GenBank under accession numbers FJ930164—FJ930926.

2.9. Phylogenetic Analysis. Alignments constructed using sequences from this work and through public databases were edited for sequence length in the ARB Edit 4 alignment tool and viewed as phylogenetic trees using the neighbor-joining algorithm in the ARB-parsimony tool. Branch lengths were derived from distance matrices generated from primary structures using a 16S structural mask and *E. coli* numbering filter. ARB-parsimony allowed sequences to be added, merged, and/or removed from trees without major recalculation of the original tree. 1000 bootstrap resamplings of the neighbor-joining data evaluated topologies of each subtree. Bootstrap values were placed at the respective nodes, with the exception of values of 100%. Algorithms used are available in the ARB sequence analysis software [26].

2.10. Statistical Analysis of Microbial Communities. To investigate how similar *Bacteria* communities were in the five randomly selected quadrats along the line transect, three multivariate analyses (principal component analysis (PCA), principal coordinate analysis (PCO), and cluster analysis (CA)) were conducted on a binary presence/absence matrix denoting if an OTU was present (+) or not (−) in a quadrat. All calculations and graphical representations were completed in the R statistical environment using the “vegan” and “Biodiversity R” packages [27, 28].

3. Results and Discussion

Bacteria in mucus collected from *P. compressa* and *P. lobata* and from adjacent seawater in five quadrats along a line transect on a shallow reef off Maui were investigated through 16S rDNA clone libraries. Physical and chemical parameters in seawater varied little in the four days prior to collection of experimental mucus and seawater samples, with generally trace levels of nutrients and high dissolved oxygen concentrations (Table 1). *Bacteria* 16S rDNA libraries from both *Porites* species’ mucus showed only modest OTU replication along the transect.

Some similarities in *Bacteria* communities in coral mucus from the same and different species have been reported [4, 5, 29]. However, *Bacteria* communities in the coral mucus analyzed here were only ∼8.4% similar in the same *Porites* species along the transect, 1.7% similar in both *Porites*, and 6.3% similar in seawater. *Bacteria* richness in mucus also varied between colonies of the same species in different quadrats along the transect, with Shannon diversity indices ranging from 0.0 to 3.2. Multivariate analyses (PCA, PCO, CA) indicated that the communities had little in common in terms of presence or absence of their component OTUs (data not shown).

One OTU (C7-A01c) occurred in all coral mucus libraries and one of the five seawater libraries. This OTU affiliated with sequences that together form an unclassified clade in the Oceanospirillaceae, part of the Gammaproteobacteria (Figure 1), including OTUs reported elsewhere from *Montastraea faveolata* (FJ202175, FJ202634, FJ202970) [10], *M. annularis* (DQ200446) [30], *M. franksi* (GU118838) [31], *Acropora cervicornis* (GU117995) [31] and the hindgut of the corallivorous six-bar angelfish, *Pomacanthus sexstriatus* from the Great Barrier Reef (EU884929, EU884930) (Esther Angert, pers. comm.). C7-A01c also shares 97.3% nucleotide identity with OTU PA1 from *P. astreoides* and *P. compressa* [4]; PA1 falls in the same clade as C7-A01c when they are aligned with their 48 nearest neighbors and 15 nearest type strains (Figure 1).

Two Oceanospirillales genera whose members occur in habitats similar to those in which we detected C7-A01c are *Endozoicomonas* and *Oceanospirillum*. The *Endozoicomonas* genus type strain was isolated from the sea slug *Elysia ornata* [32] and, since then, *E. montiporae* has been described from an encrusting *Montipora* coral in Taiwan [33]. Uncharacterized *Endozoicomonas* spp. have also been reported from the soft coral *Muricea elongata* (DQ917901) and sea cucumber *Apostichopus japonicus* (FJ357696). *Oceanospirillum* spp. include obligately heterotrophic rods that are known biofilm producers that enable other bacteria to colonize surfaces [34]. However, OTU C7-A01c and its affiliates form a separate and distinct clade.

This work and data published by others show that a single clade in the Oceanospirillaceae occurs in *Porites* spp. and other hermatypic corals from Australia, Hawai‘i, and Bermuda, suggesting a previously unreported, and as yet undefined association may exist between these *Bacteria* and corals, and organisms that occur on or feed on the corals [4, 10, 31, 32, 35]. While the nature of such an association

Table 1: Five-day physical parameters and inorganic nutrient concentrations along the Maui reef line transect. Samples for physical and chemical analyses were collected daily along the transect for 5 days, including the four days prior to that upon which coral mucus was collected. Concentrations of each nutrient were generally similar at each of the ten quadrats, except that ammonia concentrations increased on day 2 at blocks 1–9. Each measurement was performed in triplicate.

Quadrat	O_2 μM	O_2 ppm	pH	Salinity	Temperature °C	Turbidity NTU[a]	PO_4 μM	PO_4 mg/L	NO_3[b] μM	NO_3[b] mg/L	NH_3[c] μM	NH_3[c] mg/L
(1) Mean	492	7.88	7.92	35.8	25.2	0.89	0.13	0.01	2.10	0.13		
Range	(473–529)	(7.56–8.47)	(7.73–8.07)	(35-36)	(24.6–25.8)	(0–3.99)	(0–0.32)	(0.00–0.03)	—	—	61.76	1.03
SD[d]	23.05	0.37	0.12	0.4	0.6	1.73	0.14	0.01	—	—		
(2) Mean	507	8.10	7.88	36.0	25.2	1.33	0.08	0.1	—	—		
Range	(464–559)	(7.42–8.95)	(7.84–7.94)	(35–37)	(24.6–25.8)	(0.05–6.22)	(0–0.21)	(0.00–0.02)	—	—	59.41	1.01
SD	38.26	0.61	0.04	0.7	0.6	2.73	0.09	0.01	—	—		
(3) Mean	514	8.23	8	36.2	25.3	1.23	0.11	0.01	—	—		
Range	(479–561)	(7.67–8.97)	(7.88–8.16)	(36-37)	(24.6–25.9)	(0.2–5.16)	(0–0.21)	(0.00–0.02)	—	—	7.65	0.13
SD	32.03	0.51	0.12	0.4	0.6	2.2	0.07	0.01	—	—		
(4) Mean	536	8.57	7.94	35.8	25.6	1.13	0.11	0.01	—	—		
Range	(504–552)	(8.07–8.8)	(7.83–8.04)	(35-36)	(24.8–26.5)	(0–5.2)	(0–0.21)	(0.00–0.02)	—	—	52.94	0.90
SD	20.26	0.32	0.1	0.4	0.7	2.3	0.11	0.01	—	—		
(5) Mean	546	8.73	7.96	35.8	25.6	1.56	0.15	0.01	—	—		
Range	(501–567)	(8.02–9.07)	(7.92–8.02)	(35-36)	(24.9–26.4)	(0–7.1)	(0.11–0.21)	(0.01-0.02)	—	—	60.00	1.02
SD	26.59	0.43	0.04	0.4	0.6	3.15	0.06	0.01	—	—		
(6) Mean	535	8.56	7.92	35.8	25.7	1.98	0.19	0.02	—	—		
Range	(484–569)	(7.74–9.10)	(7.83–8.06)	(35-36)	(25.0–26.5)	(0–8.51)	(0.11–0.20)	(0.01-0.02)	—	—	17.06	0.29
SD	34.27	0.55	0.09	0.4	0.6	3.68	0	0	—	—		
(7) Mean	534	8.55	7.76	36.0	25.9	1.36	0.15	0.01	—	—		
Range	(526–557)	(8.41–8.91)	(7.23–7.95)	—	(25.1–26.5)	(0.07–5.43)	(0–0.32)	(0.00–0.03)	—	—	8.82	0.15
SD	13.21	0.21	0.3	—	0.6	2.31	0.12	0.01	—	—		
(8) Mean	527	8.43	8.03	36.1	26.0	1.97	0.15	0.01	—	—		
Range	(501–558)	(8.02–8.90)	(7.96–8.12)	(36.0–36.5)	(25.2–26.8)	(0.25–8.09)	(0–0.32)	(0.00–0.03)	—	—	85.88	1.46
SD	22.85	0.37	0.07	0.2	0.7	3.44	0.12	0.01	—	—		
(9) Mean	528	8.45	8.00	36.0	26.3	1.84	0.11	0.01	—	—		
Range	(499–554)	(7.98–8.86)	(7.86–8.07)	—	(25.4–27.0)	(0.27–8.08)	(0.00–0.21)	(0.00–0.02)	—	—	2.35	0.04
SD	19.58	0.31	0.08	—	3.49	3.49	0.07	0.01	—	—		
(10) Mean	544	8.71	7.89	35.6	26.3	1.74	0.21	0.02	—	—		
Range	(522–561)	(8.35–8.97)	(7.82–8.05)	(35.0-36.0)	(25.4–27.0)	(0.25–7.30)	(0.11–0.32)	(0.01–0.03)	—	—	17.64	0.30
SD	16.23	0.26	0.1	0.5	0.7	3.11	0.07	0.01	—	—		

[a]Highest NTU data were registered on the last day of sampling, April 16, 2008, during a southerly swell.
[b]Data shown are based on one measurement on April 16, 2008. NO_3 concentrations on other days were below the method's detection limit.
[c]Data shown are based on one measurement on April 13, 2008. NH_3 concentrations on other days were below the method's detection limit.
[d]Standard deviation.
All NO_2 and the majority of NO_3 concentrations in seawater were below the respective method's detection limit.

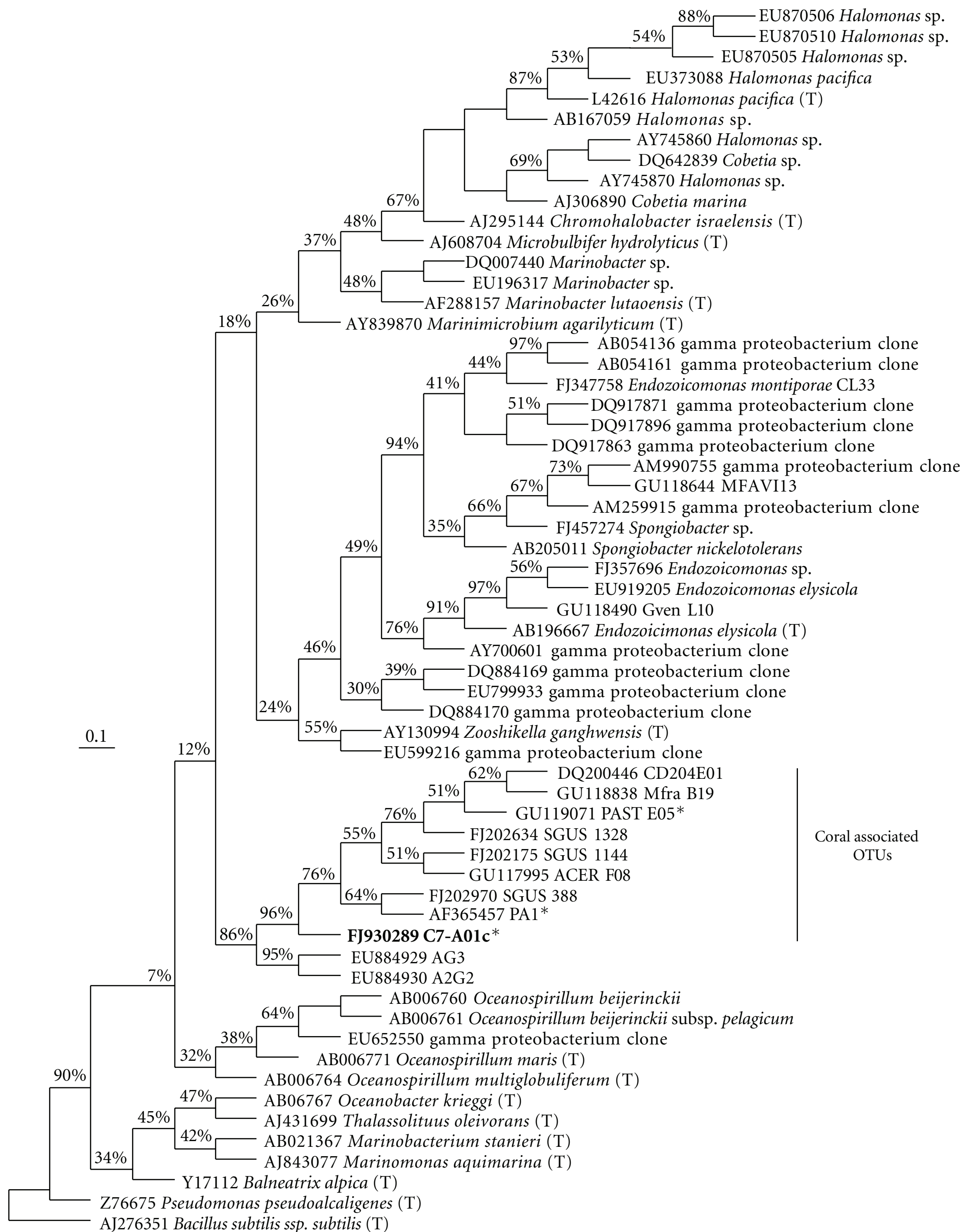

Figure 1: Neighbor-joining phylogenetic tree of OTU C7-A01c and its nearest neighbors. This OTU was detected in each *Porites* mucus sample and one adjacent seawater sample in the work described here. C7-A01c also shared at least 97.3% nucleotide identity with OTUs PA1 (AF365457) and PAST_E05 (GU119071) in *Porites astreoides* (*) from the Caribbean [4] and affiliated with clones from an uncultivated Oceanospirillaceae clade whose members include OTUs from *Montastraea faveolata* (FJ202175, FJ202634, FJ202970), *M. annularis* (DQ200446), *M. franksi* (GU118838), *Acropora cervicornis* (GU117995), and the six-bar angelfish (EU884929, EU884930). "T" designates a type strain. The tree is rooted by *Bacillus subtilis* and to 16S rRNA structural masks. Bootstrap values were generated after 1000 resamplings and are shown at the nodes. Values of 100% are not included. The scale bar represents the number of nucleotide substitutions per site, which refers to the branch lengths for comparisons between nodes.

cannot be determined through this work, Gram-negative bacteria do play an allelopathic role in some corals [36, 37]. Cultivation of members of the clade represented by OTU C7-A01c should enable their role, if any, in coral life history and perhaps in other marine invertebrates to be determined. Some speculation has centered on other *Bacteria* taxa as candidates in associations with other corals [38] but, after three decades of studies of microbes in corals, microbiologists have yet to confirm one coral-heterotrophic bacteria mutualistic interaction. In that respect, sequence-based analyses have encouraged efforts to cultivate ecologically important clades of *Bacteria* and established that some species- and genus-level phylotypes display particular distribution patterns [39, 40]. Our observation here that an Oceanospirillaceae clade may be associated with specific corals in different oceans should encourage targeted cultivation efforts; cultivating just one member of this clade will be a starting point in the elucidation of how and indeed if particular heterotrophic *Bacteria* species interact or communicate with corals or animals associated with them. Without such targets, microbiologists investigating potential *Bacteria* roles in corals may simply cultivate what they can from corals in the hope of stumbling upon a promising candidate.

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References

[1] L. Muscatine and E. Cernichiari, “Assimilation of photosynthetic products of zooxanthellae by a reef coral,” *The Biological Bulletin*, vol. 137, pp. 506–523, 1969.

[2] N. Knowlton and F. Rohwer, “Multispecies microbial mutualisms on coral reefs: the host as a habitat,” *American Naturalist*, vol. 162, no. 4, pp. S51–S62, 2003.

[3] B. E. Brown and J. C. Bythell, “Perspectives on mucus secretion in reef corals,” *Marine Ecology Progress Series*, vol. 296, pp. 291–309, 2005.

[4] F. Rohwer, V. Seguritan, F. Azam, and N. Knowlton, “Diversity and distribution of coral-associated bacteria,” *Marine Ecology Progress Series*, vol. 243, pp. 1–10, 2002.

[5] K. B. Ritchie and G. W. Smith, “Microbial communities of coral surface mucopolysaccharide layers,” in *Coral Health and Disease*, E. Rosenberg and Y. Loya, Eds., pp. 259–278, Springer, New York, NY, USA, 2004.

[6] L. Wegley, Y. Yu, M. Breitbart, V. Casas, D. I. Kline, and F. Rohwer, “Coral-associated archaea,” *Marine Ecology Progress Series*, vol. 273, pp. 89–96, 2004.

[7] T. D. Ainsworth, M. Fine, L. L. Blackall, and O. Hoegh-Guldberg, “Fluorescence in situ hybridization and spectral imaging of coral-associated bacterial communities,” *Applied and Environmental Microbiology*, vol. 72, no. 4, pp. 3016–3020, 2006.

[8] I. S. Johnston and F. Rohwer, “Microbial landscapes on the outer tissue surfaces of the reef-building coral Porites compressa,” *Coral Reefs*, vol. 26, no. 2, pp. 375–383, 2007.

[9] T. D. Ainsworth and O. Hoegh-Guldberg, “Bacterial communities closely associated with coral tissues vary under experimental and natural reef conditions and thermal stress,” *Aquatic Biology*, vol. 4, no. 3, pp. 289–296, 2008.

[10] S. Sunagawa, T. Z. Desantis, Y. M. Piceno et al., “Bacterial diversity and white Plague disease-associated community changes in the caribbean coral *Montastraea faveolata*,” *ISME Journal*, vol. 3, no. 5, pp. 512–521, 2009.

[11] M. Garren, L. Raymundo, J. Guest, C. D. Harvell, and F. Azam, “Resilience of coral-associated bacterial communities exposed to fish farm effluent,” *PLoS One*, vol. 4, no. 10, Article ID e7319, 2009.

[12] E. G. Ruby, “Lessons from a cooperative, bacterial-animal association: the *Vibrio fischeri-Euprymna scolopes* light organ symbiosis,” *Annual Review of Microbiology*, vol. 50, pp. 591–624, 1996.

[13] R. C. Schmitt, *Hawai'i Data Book*, Mutual Publishing, Honolulu, Hawaii, USA, 2002.

[14] D. Gulko, *Hawaiian Coral Reef Ecology*, Mutual Publishing, Honolulu, Hawaii, USA, 1998.

[15] S. B. Galloway, T. M. Work, and V. S. Bochsler, “Coral disease and health workshop: coral histopathology II,” NOAA Technical Memorandum NOS NCCOS 56 and NOAA Techical Memorandum CRCP, National Oceanic and Atmospheric Administration, Silver Springs, Md, USA, 2007.

[16] A. L. Reysenbach, L. J. Giver, G. S. Wickham, and N. R. Pace, “Differential amplification of ribosomal-RNA genes by polymerase chain-reaction,” *Applied and Environmental Microbiology*, vol. 58, pp. 3417–3418, 1992.

[17] W. G. Weisburg, S. M. Barns, D. A. Pelletier, and D. J. Lane, “16S ribosomal DNA amplification for phylogenetic study,” *Journal of Bacteriology*, vol. 173, no. 2, pp. 697–703, 1991.

[18] J. Sambrook, E. F. Fritsch, and T. Maniatis, Eds., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York, NY, USA, 1989.

[19] T. Huber, G. Faulkner, and P. Hugenholtz, “Bellerophon: a program to detect chimeric sequences in multiple sequence alignments,” *Bioinformatics*, vol. 20, no. 14, pp. 2317–2319, 2004.

[20] T. Z. DeSantis, P. Hugenholtz, N. Larsen et al., “Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB,” *Applied and Environmental Microbiology*, vol. 72, no. 7, pp. 5069–5072, 2006.

[21] J. R. Cole, B. Chai, R. J. Farris et al., “The ribosomal database project (RDP-II): introducing myRDP space and quality controlled public data,” *Nucleic Acids Research*, vol. 35, no. 1, pp. D169–D172, 2007.

[22] S. F. Altschul, T. L. Madden, A. A. Schäffer et al., “Gapped BLAST and PSI-BLAST: a new generation of protein database search programs,” *Nucleic Acids Research*, vol. 25, no. 17, pp. 3389–3402, 1997.

[23] P. D. Schloss and J. Handelsman, “Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness,” *Applied and Environmental Microbiology*, vol. 71, no. 3, pp. 1501–1506, 2005.

[24] R. K. Colwell, X. M. Chang, and J. Chang, "Interpolating, extrapolating, and comparing incidence-based species accumulation curves," *Ecology*, vol. 85, no. 10, pp. 2717–2727, 2004.

[25] C. X. Mao, R. K. Colwell, and J. Chang, "Estimating the species accumulation curve using mixtures," *Biometrics*, vol. 61, no. 2, pp. 433–441, 2005.

[26] W. Ludwig, O. Strunk, R. Westram et al., "ARB: a software environment for sequence data," *Nucleic Acids Research*, vol. 32, no. 4, pp. 1363–1371, 2004.

[27] J. Oksanen, R. Kindt, and R. B. O'Hara, "Vegan: community ecology package," 2005.

[28] R Development Core Team, *R: A Language and Environment for Statistical Computing*, R Foundation for Statistical Computing, Vienna, Austria, 2005.

[29] H. Ducklow and R. Mitchell, "Bacterial populations and adaptations in the mucus layers on living corals," *Limnology and Oceanography*, vol. 24, pp. 715–725, 1979.

[30] J. S. Klaus, I. Janse, J. M. Heikoop, R. A. Sanford, and B. W. Fouke, "Coral microbial communities, zooxanthellae and mucus along gradients of seawater depth and coastal pollution," *Environmental Microbiology*, vol. 9, no. 5, pp. 1291–1305, 2007.

[31] S. Sunagawa, C. M. Woodley, and M. Medina, "Threatened corals provide underexplored microbial habitats," *PLoS One*, vol. 5, no. 3, Article ID e9554, 2010.

[32] M. Kurahashi and A. Yokota, "*Endozoicomonas elysicola* gen. nov., sp. nov., a γ-proteobacterium isolated from the sea slug Elysia ornata," *Systematic and Applied Microbiology*, vol. 30, no. 3, pp. 202–206, 2007.

[33] C. S. Yang, M. H. Chen, A. B. Arun, C. A. Chen, J. T. Wang, and W. M. Chen, "*Endozoicomonas montiporae* sp. nov., isolated from the encrusting pore coral *Montipora aequituberculata*," *International Journal of Systematic and Evolutionary Microbiology*, vol. 60, no. 5, pp. 1158–1162, 2010.

[34] B. Little, P. Wagner, P. Angell, and D. White, "Correlation between localized anodic areas and *Oceanospirillum* biofilms on copper," *International Biodeterioration and Biodegradation*, vol. 37, no. 3-4, pp. 159–162, 1996.

[35] D. G. Bourne and C. B. Munn, "Diversity of bacteria associated with the coral *Pocillopora damicornis* from the Great Barrier Reef," *Environmental Microbiology*, vol. 7, no. 8, pp. 1162–1174, 2005.

[36] K. M. E. Gnanambal, C. Chellaram, and J. Patterson, "Isolation of antagonistic marine bacteria from the surface of the gorgonian corals at Tuticorin, south east coast of India," *Indian Journal of Marine Sciences*, vol. 34, no. 3, pp. 316–319, 2005.

[37] K. B. Ritchie, "Regulation of microbial populations by coral surface mucus and mucus-associated bacteria," *Marine Ecology Progress Series*, vol. 322, pp. 1–14, 2006.

[38] V. Casas, D. I. Kline, L. Wegley, Y. Yu, M. Breitbart, and F. Rohwer, "Widespread association of a *Rickettsiales*-like bacterium with reef-building corals," *Environmental Microbiology*, vol. 6, no. 11, pp. 1137–1148, 2004.

[39] M. S. Rappé, S. A. Connon, K. L. Vergin, and S. J. Giovannoni, "Cultivation of the ubiquitous SAR11 marine bacterioplankton clade," *Nature*, vol. 418, no. 6898, pp. 630–633, 2002.

[40] M. V. Brown and S. P. Donachie, "Evidence for tropical endemicity in the *Deltaproteobacteria* Marine Group B/SAR324 bacterioplankton clade," *Aquatic Microbial Ecology*, vol. 46, no. 2, pp. 107–115, 2007.