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Phylogenetic diversity of cultured bacteria from prevalent species of corals around Samae San island, Thailand

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ABSTRACT: Predominant corals of Samae San island, Thailand, including *Acropora humilis*, *Acropora millepora*, *Porites lutea* and *Platygyra sinensis*, were cultured and identified for bacterial species by 16S rRNA gene sequencing. Of all corals, dominant cultured bacteria were Firmicutes (46.75%), Proteobacteria (34.60%), Actinobacteria (17.18%) and Bacteriodetes (1.47%). Firmicutes such as *Staphylococcus*, *Bacillus* and *Sediminibacillus* was relatively most abundant (∼50%), except in *P. sinensis* that Proteobacteria was more abundant. Over culture temperature range of 20–50 °C, different bacterial species were grown (ANOVA, $p < 0.05$). Coral *P. lutea* and *A. humilis* associated bacteria were able to be cultured at the highest temperature (45 °C), followed by coral *A. millepora* (40 °C) and *P. sinensis* (35 °C) bacteria. The high-temperature cultured bacteria were mostly *Bacillus* such as *Bacillus amyloliquefaceins*. Multiple sequence alignment and phylogeny relationship of the bacterial species from these four corals showed that, for Firmicutes and Proteobacteria, the bacterial species isolated from coral *P. lutea*, *A. humilis* and *A. millepora* rather shared clades. Overall, the coral *Acropora* demonstrated more diversity of bacterial species than coral *Porites*. The culturing attempt at high temperature allowed additional bacterial species findings.

KEYWORDS: bacterial diversity, 16S rRNA gene sequencing, coral-associated bacteria, Thailand

INTRODUCTION

Coral reefs are well-known resources to support large, biodiverse communities of organisms and microorganisms both in the marine ecosystem and on Earth. For instance, coral reefs function as shelters for many lives and are nutrient-rich habitat. Coral-associated bacteria were reported either on a coral's surface, a calcium carbonate skeleton, or its inside hollow [1, 2]. The co-living between corals and bacteria shares mutual benefits, including but not limited to, exchange of nutrients (e.g., corals provide nutrient-rich resources while bacteria are initial producers and final recyclers of organic food chains) and support of coral resistance to high temperature and pathogen (e.g., bacterial antioxidants and secondary metabolites) [3, 4]. Hence, specific bacteria were reported to correlate with disease-resistant corals [3, 5–7], yet study on cultivation of the coral-associated bacteria from prevalent species of corals in Samae San island, Thailand, the region where coral reefs are abundant [8], had been limited. Samae San island locates in the east Gulf of Thailand. This study cultured and identified coral bacteria in all prevalent species of corals in Samae San island including *Acropora humilis*, *Acropora millepora*, *Porites lutea* and *Platygyra sinensis*, and analyzed their 16S rRNA gene sequence alignment and phylogenetic relationship. Additionally, as global warming (thus rising water temperature) is a cause of coral bleaching worldwide during the last decade, we as well described the cultured coral-associated bacteria that could survive at relatively high temperature (up to 50 °C). This supports the better understanding of the bacterial diversity and coral species during high temperature, and the collection of live bacterial isolates are also important for future physiological studies.

Previously, culture and culture-independent (metagenomics) studies reported that coral-

associated bacteria are sometimes specific to coral species and environments [1, 9, 10]. Given cultivation allows to obtain live bacteria collection for physiological and functional studies [11, 12] and no bacteria collection associated with coral species and high temperature had been cultivated before in Samae San island coral reefs, thus signifying the bacteria collection in this study.

MATERIALS AND METHODS

Sample collections

Healthy corals of *A. humilis*, *A. millepora*, *P. lutea* and *P. sinensis* were collected around Samae San island on April 28, 2014 (Fig. 1). Of each coral specie, 3 independent samples of 2 cm diameter size were collected in separate sterile bags and transported on ice immediately to laboratory.

Cultivation of bacteria

The protocols of cultivation followed established protocols [13] with some modifications. Each coral sample was ground and 1 g was serially diluted from 10^{-1}–10^{-5} with 0.2 μm filtered-sterile seawater. Each dilution (100 μl) was spread onto a seawater nutrient agar (SNA; autoclaved seawater supplemented with 0.3% beef extract and 0.5% peptone in 1.5% agar) and incubated under aerobic condition at 20, 25, 30, 35, 40, 45 and 50 °C for 2 weeks. Hence, each set of experiment consisted of 5 serial dilutions, 7 incubation conditions and 2 independent replicate sets. Total colony count represents the number of cultured bacteria in colony forming unit (CFU) per g of coral. Then, colonies of different morphologies were transferred to another SNA for streaking to isolate colonies. Gram stain and optical microscopic record were performed on isolated colonies for confirmation.

16S rRNA gene amplification and sequencing

Single colony from each isolate was picked into a 25-μl PCR reaction, comprising 1 × EmeraldAmp GT PCR Master Mix (TaKaRa, Shiga, Japan), 0.3 μM of each of the forward and reverse primers and template (single colony or 50 ng of DNA). If colony PCR did not yield result, the isolate colony was cultured in SN broth, and the bacterial genome was extracted using GF1-Bacterial DNA Extraction Kit (Vivantis, California, USA). The extracted DNA was determined for the concentration and the purity using nanodrop spectrophotometer, and 50 ng of the DNA was used in PCR. Full-length 16S rRNA gene is amplified using universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) [14, 15]. The PCR thermocycling conditions were 95 °C 4 min, 30–35 cycles of 94 °C 1 min, 55 °C 1 min and 72 °C 2 min, followed by 72 °C 10 min. An amplicon corresponding to the full-length 16S rRNA gene (1500

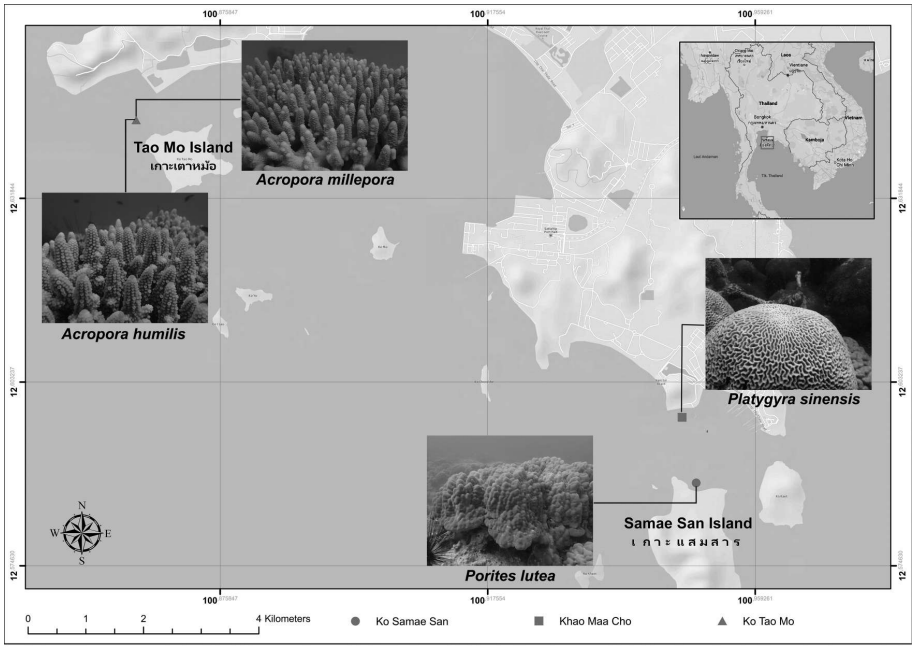

Fig. 1 Photograph of coral samples and study sites.

base pairs) was visualized by 1.5% agarose gel electrophoresis, purified and sequenced using Sanger's sequencer ABI3730XL at Macrogen Inc., Korea.

Bacteria species identification, multiple sequence alignment, and phylogenetic analysis

Ambiguous nucleotides on both ends of raw sequences were trimmed by manual inspection of electropherographs showing mixed peaks of nucleotides. Trimmed sequences were identified for species by BLASTN against NCBI non-redundant database with $\leqslant 10^{-4}$ E-value cutoff, unless specified otherwise [16]. The cultured bacterial sequences and reference sequences were also aligned using CLUSTALX [17], and a neighbor joining tree with 1000 bootstrap replicates was constructed using MEGA version 7 [18], in order to get the glimpse on the putative distance between the cultured bacterial species and the references. A correlation between coral species and a temperature in which the different bacterial diversity were cultured was determined by ANOVA statistic (p values).

RESULTS

Total colony count

The number of cultured colonies in CFUs (0.6–3.8×10^3 CFU/g), including the number of different colony morphologies (7–27 cultured bacterial IDs), varied by both coral species and culture temperatures. *A. humilis* and *P. lutea* showed the highest total bacterial count, followed by *P. sinensis* and *A. millepora*, respectively. The number of different morphologies (cultured bacterial diversity) followed the same order as the total bacterial count, which is *A. humilis*, *P. lutea*, *P. sinensis* and *A. millepora* (Table 1). For culture temperature, 30–35 °C allowed the maximum total bacterial counts and the number of bacterial diversity. *P. sinensis* and *A. millepora* demonstrated the relatively narrow temperature growth range whereas *A. humilis* and *P. lutea* could be cultured at up to 45 °C (0.1–0.4×10^3 CFU/g coral and 1–2 different colony morphologies) (Table 1).

Identification of bacterial species

Unique colony morphologies of each coral species were analyzed by gram stain (gram-positive or gram-negative) and optical microscopy to confirm distinct cell morphology. The confirmed distinct isolates with at least 9% relative abundance were sequenced and identified for species using BLASTN (Table S1). Most bacterial species belonged to phyla

Table 1 Total colony counts in CFUs/g coral and the number of different morphology colonies from four coral species cultured at different temperatures.

Sample	Temp. (°C)	CFU/g	No. isolate
A. humilis	20	0	0
	25	2200	9
	30	3400	17
	35	2700	14
	40	1100	4
	45	100	1
	50	0	0
A. millepora	20	0	0
	25	500	4
	30	600	5
	35	500	7
	40	100	1
	45	0	0
	50	0	0
P. lutea	20	0	0
	25	2700	27
	30	2600	22
	35	3200	27
	40	700	4
	45	400	2
	50	0	0
P. sinensis	20	0	0
	25	1900	6
	30	1900	6
	35	3800	11
	40	0	0
	45	0	0
	50	0	0

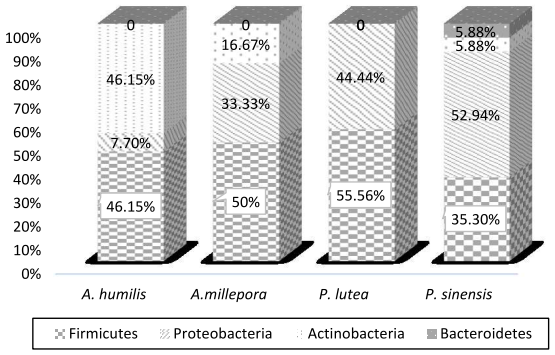

Fig. 2 Percent distribution of cultured bacteria at phylum level.

Firmicutes (35.30–46.15%), Proteobacteria (7.70–52.94%) and Actinobacteria (0–46.15%), except *P. sinensis* that also contain species in phylum Bacteriodetes for 5.88% (Fig. 2). Calculating the diversity index via phylum richness and phylum evenness (a

relative distribution of each phylum) showed that *A. millepora* and *P. sinensis* provided the highest bacterial diversity, although the minimal number of cultured bacterial IDs was from *A. millepora* (6 different species) (Table S1). For differences in the cultured temperatures, ANOVA statistic indicated an association between temperature and the bacterial diversity ($p < 0.05$).

In addition, the cultured bacteria and the reference sequences were aligned to observe pairwise distances among sequences (Table S2). In Firmicutes, the cultured bacteria from *P. sinensis* showed a relatively separate clade compared with those in the other three coral species, which were clustered at 76% confidence. Shared bacterial species among these three coral species included unidentified species of *Staphylococcus* (i.e., *Staphylococcus* sp.), *Staphylococcus epidermidis* and *Bacillus amyloliquefaciens* (Fig. S1a). For bacteria in phyla Proteobacteria and Actinobacteria, the *P. sinensis*-associated bacteria showed overlapped similarities (Table S2: smaller number of base substitutions and Fig. S1b: shared clades of trees). Nevertheless, sequences in this study were from a single sequencing reaction (700 base pairs), so the alignment and the phylogenetic relatedness compared with the reference sequences remained elusive.

DISCUSSION

Diverse bacterial communities were reported on coral reefs worldwide [2, 19]. This report represented the first cultured coral-associated bacteria diversity from prevalent coral species (*A. humilis*, *A. millepora*, *P. lutea* and *P. sinensis*) in Samae San island. Different coral species contained diversity and frequencies of bacteria (2750 CFUs/g coral), and bacteria from certain coral species were able to survive and grow at high temperature (i.e., *A. humilis* and *P. lutea*). These bacteria could be important for future physiological studies that involve temperature resistance.

Most cultured bacteria were gram-positive, supporting a general gram stain type reported in marine elsewhere [13] because gram-positive bacteria cell membrane with thick peptidoglycan layer allow bacteria to be more resistant than gram-negative bacteria cell membrane. Identified species of Firmicutes were capable of forming endospore (i.e., *Bacillus* sp.), which is a resistant form of bacteria to a harsh environment such as hot and dry. Additionally, *Bacillus* and Actinobacteria species are producers of antimicrobial compounds such as bacitracin and polymyxin B that might help protect corals from pathogenic bacteria [10, 20, 21]. Supportively, Liang et al [22] reported that *Acropora* had the relatively high bacteria diversity, yet coral pathogen bacteria detected, thus highlighting a relationship between this coral-associated bacteria community with the coral resistance to pathogens.

However, our reported diversity of cultured bacteria was far fewer than that published by metagenomics combined next generation sequencing (culture-independent) methods, although the finding of high prevalence of proteobacteria remains common [19, 23]. The far fewer number by cultured method was consistent with many publications that compared the cultured and culture-independent methods [13].

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2020.018.

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Appendix A. Supplementary data

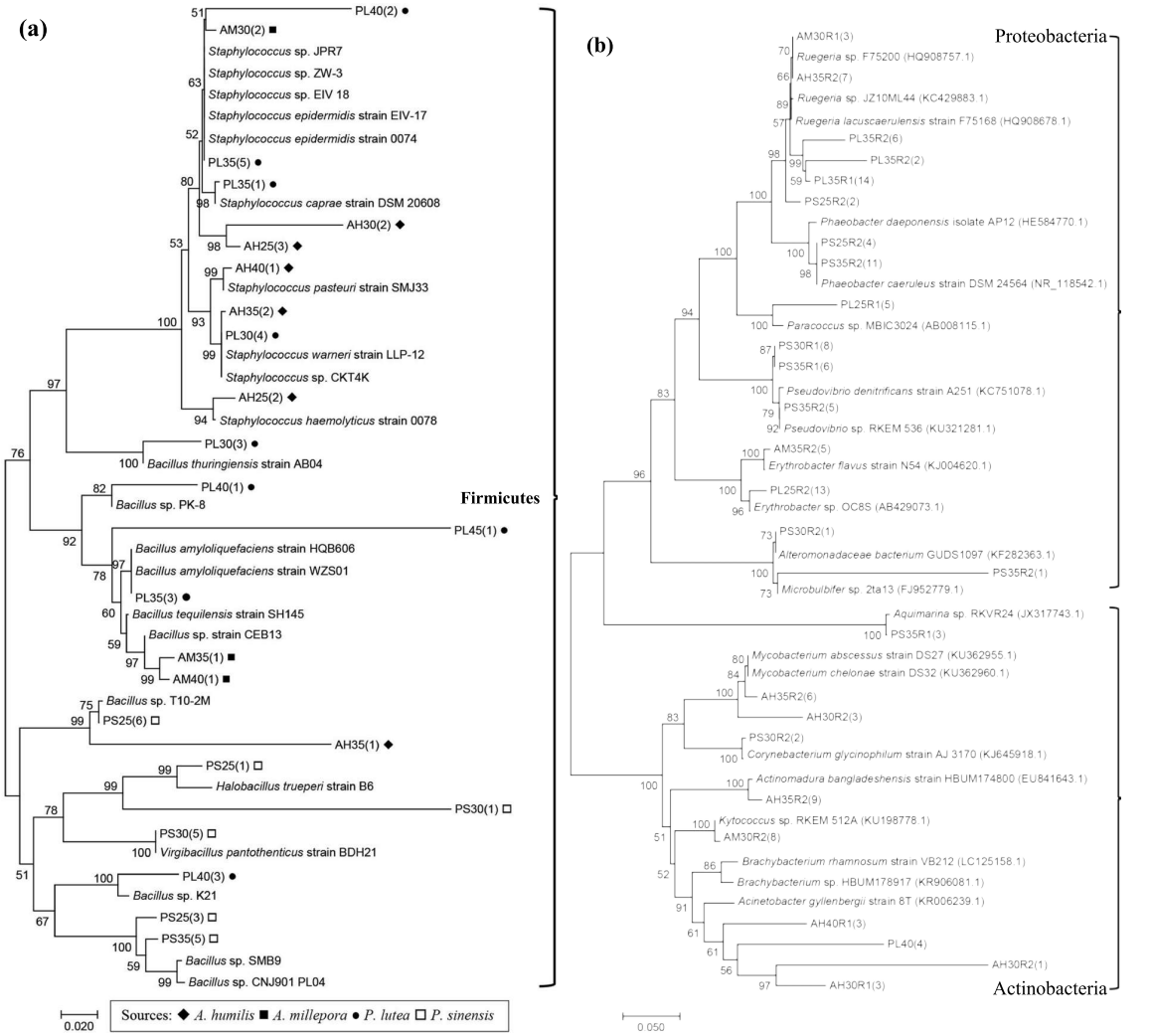

Fig. S1 Neighbor-joining tree of cultured bacterial IDs and their reference species in phyla (a) Firmicutes and (b) Proteobacteria, Actinobacteria and Bacteroidetes. Number at node represents percent bootstrap replicates (cluster confidence), and < 50% bootstrap replicates were not displayed. A bar at a bottom of the tree represents a unit of genetic distance.

Table S1 Species and phylum identification by BLASTN.

ID	GenBank No.	Species identification	Nucleotide (%)	Phylum
***A. humilis* coral**				
AH25(1)	KP236214.1	*Staphylococcus haemolyticus* strain 0078	100	Firmicutes
AH25(2)	KU821699.1	*Staphylococcus warneri* strain LLP-12	99	Firmicutes
AH25(3)	KU323914.1	*Staphylococcus* sp. ZW-3	100	Firmicutes
AH30(1)	LC125158.1	*Brachybacterium rhamnosum* strain: VB-2.1.2	99	Actinobacteria
AH30(2)	KR906081.1	*Brachybacterium* sp. HBUM178917	95	Actinobacteria
AH30(3)	KU362955.1	*Mycobacterium abscessus* strain DS27	97	Actinobacteria
AH35(1)	AB617555.1	*Bacillus* sp. T10-2M	91	Firmicutes
AH35(2)	KU821699.1	*Staphylococcus warneri* strain LLP-12	100	Firmicutes
AH35(3)	KU362960.1	*Mycobacterium chelonae* strain DS32	99	Actinobacteria
AH35(4)	HQ908757.1	*Ruegeria* sp. F75200	99	Proteobacteria
AH35(5)	EU841643.1	*Actinomadura bangladeshensis* strain HBUM174800	99	Actinobacteria
AH40(1)	KT036409.1	*Staphylococcus pasteuri* strain SMJ33	99	Firmicutes
AH40(2)	LT547841.1	*Micrococcus yunnanensis* isolate 54 SP633	95	Actinobacteria
***A. millepora* coral**				
AM30(1)	HQ908678.1	*Ruegeria lacuscaerulensis* strain F75168	99	Proteobacteria
AM30(2)	FJ613575.1	*Staphylococcus epidermidis* strain EIV-17	99	Firmicutes
AM30(3)	KU198778.1	*Kytococcus* sp. RKEM 512A	98	Actinobacteria
AM35(1)	KC172053.1	*Bacillus tequilensis* strain SH145	98	Firmicutes
AM35(2)	KJ004620.1	*Erythrobacter flavus* strain N54	99	Proteobacteria
AM40(1)	KX681803.1	*Bacillus* sp. strain CEB13	98	Firmicutes
***P. lutea* coral**				
PL25(1)	AB008115.1	*Paracoccus* sp. MBIC3024	99	Proteobacteria
PL25(2)	HQ288801.1	*Erythrobacter nanhaisediminis* strain F75112	99	Proteobacteria
PL25(3)	AB429073.1	*Erythrobacter* sp. OC8S	99	Proteobacteria
PL30(1)	HQ439523.1	*Ruegeria* sp. MR31c	100	Proteobacteria
PL30(2)	FJ613576.1	*Staphylococcus* sp. EIV-18	96	Firmicutes
PL30(3)	KX245016.1	*Bacillus thuringiensis* strain AB04	100	Firmicutes
PL30(4)	KU051664.1	*Staphylococcus* sp. CKT4K	100	Firmicutes
PL35(1)	NR_119252.1	*Staphylococcus caprae* strain DSM 20608	99	Firmicutes
PL35(2)	HQ908705.1	*Ruegeria lacuscaerulensis* strain F77045	99	Proteobacteria
PL35(3)	KX665550.1	*Bacillus amyloliquefaciens* strain WZS01	99	Firmicutes
PL35(4)	KU560505.1	*Ruegeria* sp. LMB	98	Proteobacteria
PL35(5)	KP236210.1	*Staphylococcus epidermidis* strain 0074	99	Firmicutes
PL35(6)	KC429803.1	*Ruegeria* sp. JZ10IS2	95	Proteobacteria
PL40(1)	EU685817.1	*Bacillus* sp. PK-8	96	Firmicutes
PL40(2)	KM083802.1	*Staphylococcus* sp. JPR7	92	Firmicutes
PL40(3)	KT200230.1	*Bacillus* sp. K21	98	Firmicutes
PL40(4)	KR006239.1	*Acinetobacter gyllenbergii* strain 8T	99	Proteobacteria
PL45(1)	KX155823.1	*Bacillus amyloliquefaciens* strain HQB606	98	Firmicutes
***P. sinensis* coral**				
PS25(1)	FJ157159.1	*Halobacillus trueperi* strain B6	98	Firmicutes
PS25(2)	KC429883.1	*Ruegeria* sp. JZ10ML44	99	Proteobacteria
PS25(3)	DQ868675.1	*Bacillus* sp. SMB9	99	Firmicutes
PS25(4)	FJ161368.1	*Ruegeria atlantica* strain D7087	94	Proteobacteria
PS25(5)	HE584770.1	*Phaeobacter daeponensis* isolate AP12	99	Proteobacteria
PS25(6)	AB617555.1	*Bacillus* sp. T10-2M	99	Firmicutes
PS30(1)	KM010131.1	*Sediminibacillus halophilus* strain muz2b	87	Firmicutes
PS30(2)	KC751078.1	*Pseudovibrio denitrificans* strain A-25	98	Proteobacteria
PS30(3)	KF282363.1	*Alteromonadaceae bacterium* GUDS1097	98	Proteobacteria
PS30(4)	KJ645918.1	*Corynebacterium glycinophilum* strain AJ 3170	96	Actinobacteria
PS30(5)	KF933616.1	*Virgibacillus pantothenticus* strain BDH21	99	Firmicutes
PS35(1)	HQ908670.1	*Ruegeria lacuscaerulensis* strain F71078	99	Proteobacteria
PS35(2)	JX317743.1	*Aquimarina* sp. RKVR24	99	Bacteroidetes
PS35(3)	FJ952779.1	*Microbulbifer* sp. 2ta13	98	Proteobacteria
PS35(4)	KU321281.1	*Pseudovibrio* sp. RKEM 536	99	Proteobacteria
PS35(5)	DQ448795.1	*Bacillus* sp. CNJ901 PL04	98	Firmicutes
PS35(6)	NR_118542.1	*Phaeobacter caeruleus* strain DSM 24564	99	Proteobacteria

Of each coral species, data were ordered by cultured temperature and within the same temperature, data were ordered by relative abundance. The first 2 letters of cultured bacterial ID (ID) represent coral species, the following number is the lowest cultured temperature that the colony was found, and the number in parenthesis represents a dilution where the colony was taken from. Only distinct morphology colonies with at least 9% relative abundance on individually cultured SNA plates were sequenced, and only cultured bacterial IDs that received the significant E-value cutoffs by BLASTN were displayed.

Table S2 Comparison of pairwise nucleotide differences among isolated bacteria and reference 16S rRNA partial gene sequences of (a) Firmicutes and (b) Proteobacteria, Actinobacteria and Bacteroidetes.

(a)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
2	.39																																																
3	.37	.04																																															
4	.36	.04	.04																																													
5	.49	.61	.61	.56																																												
6	.36	.47	.47	.42	.25																																											
7	.38	.50	.50	.40	.26	.18																																										
8	.40	.52	.52	.41	.27	.19	.21																																								
9	.38	.51	.51	.39	.25	.18	.17	.18																																							
10	.44	.58	.58	.55	.42	.36	.32	.31	.29																																						
11	.33	.42	.42	.38	.30	.19	.17	.21	.19	.30																																					
12	.37	.50	.49	.46	.33	.22	.19	.19	.18	.28	.15																																				
13	.47	.53	.53	.51	.37	.24	.24	.23	.20	.25	.20	.17																																			
14	.42	0.53	.51	.46	.36	.24	.22	.23	.19	.21	.18	.14	.09																																		
15	.44	.54	.54	.42	.38	.28	.24	.28	.27	.35	.25	.20	.31	.26																																	
16	.36	.46	.47	.44	.33	.23	.20	.20	.19	.34	.18	.18	.25	.19	.14																																
17	.36	.46	.47	.44	.33	.23	.20	.20	.19	.34	.18	.18	.25	.19	.14	.00																															
18	.37	.48	.47	.45	.34	.24	.20	.20	.19	.30	.19	.19	.24	.21	.12	.10	.10																														
19	.44	.53	.54	.51	.37	.30	.26	.26	.26	.26	.25	.24	.22	.19	.19	.15	.15	.14																													
20	.27	.36	.35	.36	.31	.21	.20	.24	.24	.29	.15	.16	.21	.17	.16	.08	.08	.07	.13																												
21	.28	.37	.36	.36	.32	.22	.21	.25	.24	.30	.16	.17	.23	.21	.17	.10	.10	.09	.15	.01																											
22	.29	.37	.36	.36	.32	.21	.21	.24	.24	.29	.15	.16	.21	.18	.17	.09	.09	.07	.14	.00	.01																										
23	.28	.37	.37	.36	.26	.17	.13	.14	.13	.28	.12	.14	.22	.18	.06	.07	.07	.07	.13	.00	.01	.00																									
24	.32	.43	.43	.41	.28	.16	.14	.13	.13	.26	.13	.14	.23	.19	.07	.04	.04	.07	.14	.01	.03	.01	.01																								
25	.35	.45	.45	.42	.34	.22	.17	.18	.17	.23	.16	.16	.21	.18	.12	.07	.07	.07	.07	.04	.07	.05	.05	.06																							
26	.35	.44	.44	.42	.32	.22	.17	.17	.17	.23	.16	.16	.22	.17	.11	.07	.07	.08	.09	.05	.07	.05	.05	.06	.04																						
27	.28	.38	.37	.36	.31	.22	.21	.24	.24	.28	.15	.17	.22	.18	.18	.09	.09	.09	.12	.01	.02	.02	.02	.02	.03	.03																					
28	.32	.44	.44	.42	.30	.19	.16	.15	.15	.24	.15	.16	.22	.18	.09	.05	.05	.08	.12	.03	.04	.03	.03	.03	.06	.07	.04																				
29	.32	.42	.41	.39	.30	.21	.16	.18	.17	.30	.16	.18	.23	.19	.10	.10	.10	.08	.15	.04	.05	.04	.01	.02	.06	.07	.05	.04																			
30	.27	.37	.36	.35	.31	.21	.17	.18	.18	.29	.12	.17	.22	.18	.07	.09	.09	.08	.14	.00	.01	.00	.00	.01	.05	.06	.02	.03	.04																	
31	.36	.45	.45	.44	.31	.18	.17	.15	.14	.23	.13	.12	.06	.08	.23	.18	.18	.21	.18	.16	.16	.16	.17	.18	.15	.16	.17	.19	.18	.17																
32	.31	.40	.39	.36	.30	.21	.21	.24	.23	.28	.13	.14	.14	.10	.27	.19	.19	.19	.24	.12	.12	.12	.12	.14	.15	.16	.12	.16	.16	.12	.06															
33	.28	.36	.34	.34	.31	.19	.19	.23	.22	.28	.12	.09	.17	.14	.24	.16	.16	.17	.23	.08	.08	.08	.09	.10	.15	.14	.10	.13	.13	.08	.10	.06														
34	.35	.46	.46	.41	.29	.15	.13	.13	.10	.21	.10	.08	.12	.06	.19	.13	.13	.15	.19	.13	.15	.13	.13	.13	.15	.14	.12	.14	.14	.13	.06	.05	.08													
35	.30	.41	.41	.37	.24	.12	.09	.11	.09	.26	.08	.09	.12	.08	.15	.12	.12	.17	.23	.09	.11	.09	.09	.11	.14	.14	.09	.13	.10	.09	.07	.04	.05	.03												
36	.28	.37	.36	.34	.29	.18	.18	.22	.21	.27	.12	.12	.13	.11	.25	.16	.16	.17	.23	.09	.09	.09	.10	.11	.15	.14	.09	.13	.14	.09	.06	.03	.04	.04	.02											
37	.28	.37	.35	.35	.28	.18	.18	.22	.21	.27	.11	.11	.12	.08	.25	.15	.15	.17	.22	.09	.09	.09	.09	.10	.14	.13	.09	.13	.13	.09	.06	.03	.03	.02	.00	.01										
38	.39	.52	.52	.49	.31	.22	.20	.16	.16	.26	.17	.17	.25	.22	.21	.17	.17	.17	.22	.15	.17	.15	.15	.16	.18	.15	.13	.17	.17	.16	.19	.16	.17	.17	.16	.15	.14									
39	.27	.37	.35	.37	.30	.19	.18	.22	.22	.21	.12	.15	.18	.15	.23	.15	.15	.15	.20	.08	.08	.08	.08	.09	.11	.11	.08	.10	.12	.08	.13	.09	.07	.10	.07	.07	.07	.07	.14							
40	.25	.36	.36	.35	.30	.19	.19	.23	.21	.28	.12	.15	.21	.18	.25	.15	.15	.16	.23	.09	.09	.09	.09	.11	.15	.14	.10	.13	.13	.08	.16	.09	.07	.12	.08	.07	.07	.15	.06							
41	.27	.39	.38	.35	.27	.18	.13	.24	.22	.31	.14	.19	.24	.22	.27	.20	.20	.20	.26	.11	.13	.12	.11	.14	.17	.17	.12	.16	.15	.10	.17	.12	.10	.14	.10	.10	.09	.20	.08	.08						
42	.27	.39	.38	.37	.24	.16	.19	.23	.23	.33	.15	.19	.25	.24	.28	.21	.21	.22	.27	.11	.13	.13	.13	.16	.19	.19	.13	.18	.16	.12	.18	.13	.11	.17	.11	.10	.10	.21	.10	.10	.08					
43	.27	.39	.39	.37	.22	.07	.11	.14	.12	.33	.12	.18	.24	.23	.19	.18	.18	.22	.29	.14	.15	.14	.14	.16	.21	.21	.14	.19	.15	.14	.18	.13	.11	.14	.11	.10	.09	.22	.11	.11	.08	.07				
44	.30	.40	.40	.35	.29	.17	.16	.11	.13	.30	.14	.18	.22	.20	.20	.18	.18	.19	.25	.13	.14	.13	.12	.13	.18	.17	.14	.15	.16	.13	.15	.13	.11	.12	.09	.10	.10	.15	.12	.11	.12	.13	.10			
45	.28	.37	.35	.35	.28	.18	.19	.21	.19	.28	.12	.15	.18	.17	.26	.16	.16	.18	.24	.09	.09	.09	.10	.12	.15	.14	.10	.13	.14	.10	.10	.08	.06	.10	.06	.06	.05	.14	.06	.06	.10	.10	.10	.10		
46	.26	.36	.35	.35	.28	.17	.17	.22	.20	.28	.06	.15	.17	.15	.25	.16	.16	.17	.24	.09	.09	.09	.10	.12	.15	.14	.09	.13	.13	.09	.12	.07	.06	.08	.05	.05	.05	.15	.06	.05	.07	.08	.09	.10	.05	
47	.26	.37	.36	.35	.28	.17	.19	.20	.13	.28	.13	.16	.19	.17	.27	.18	.18	.19	.25	.11	.12	.12	.12	.13	.16	.16	.12	.15	.15	.11	.13	.10	.10	.10	.08	.08	.08	.15	.09	.07	.09	.09	.09	.09	.06	.06
48	.26	.36	.36	.35	.29	.17	.19	.16	.18	.29	.12	.16	.19	.19	.26	.18	.18	.19	.25	.10	.11	.11	.11	.13	.16	.16	.11	.14	.15	.10	.14	.09	.09	.11	.09	.07	.08	.14	.08	.07	.08	.09	.10	.04	.07	.06
49	.28	.35	.35	.35	.30	.19	.20	.17	.19	.31	.14	.18	.21	.22	.28	.19	.19	.21	.27	.11	.10	.10	.12	.14	.19	.18	.11	.16	.16	.11	.14	.10	.08	.14	.10	.07	.07	.16	.08	.08	.10	.10	.11	.04	.06	.06

1, #Ruegeria_sp_F75200; 2, #Aquimarina_sp_RKVR24; 3, #Flavobacteriaceae_bacterium_04OKA1712; 4, #Marine_bacterium_HE29; 5, #PS30R1_3; 6, #PS25R1_1; 7, #PS30R2_5; 8, #PS25R2_1; 9, #PS25R2_6; 10, #PL30R2_4; 11, #PL40_3; 12, #PL40_1; 13, #AM35R1_4; 14, #PL45_2; 15, #PL40_2; 16, #AH25R2_2; 17, #AH35R1_2; 18, #AH25R2_6; 19, #AH40R1_1; 20, #Staphylococcus_sp_JPR7; 21, #Staphylococcus_caprae_strain_DSM_20608; 22, #Staphylococcus_sp_ZW3; 23, #Staphylococcus_epidermidis_strain_0074; 24, #PL35R2_4; 25, #AH35R1_8; 26, #PL30R2_13; 27, #Staphylococcus_sp_CKT4K; 28, #PL35R1_5; 29, #AM30R2_7; 30, #Staphylococcus_epidermidis_strain_EIV17; 31, #AM40_1; 32, #Bacillus_sp_strain_CEB13; 33, #Bacillus_sp_PK8; 34, #PL35R1_18; 35, #Bacillus_amyloliquefaciens_strain_HQB606; 36, #Bacillus_tequilensis_strain_SH145; 37, #Bacillus_amyloliquefaciens_strain_WZS01; 38, #PL30R2_12; 39, #Bacillus_thuringiensis_strain_AB04; 40, #Bacillus_sp_H2k; 41, #Virgibacillus_pantothenticus_strain_BDH21; 42, #Sediminibacillus_halophilus_strain_muz2b; 43, #Halobacillus_trueperi_strain_B6; 44, #PS35R2_8; 45, #Bacillus_sp_SA1_12; 46, #Bacillus_sp_K21; 47, #Bacillus_sp_T10_2M; 48, #Bacillus_sp_SMB9; 49, #Bacillus_sp_CNJ901_PL04.

Table S2 (Continue.)

(b)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
2	.04																																																	
3	.68	.69																																																	
4	.62	.42	.58																																															
5	.56	.50	.49	.38																																														
6	.39	.44	.45	.22	.28																																													
7	.37	.42	.45	.32	.18	.10																																												
8	.51	.50	.45	.16	.13	.19	.06																																										
9	.57	.62	.36	.48	.39	.30	.30	.35																																									
10	.62	.58	.34	.53	.39	.40	.39	.30	.34																																								
11	.67	.62	.72	.40	.42	.40	.34	.38	.61	.46																																								
12	.52	.48	.42	.41	.35	.29	.29	.29	.30	.34	.45																																							
13	.42	.46	.40	.21	.24	.16	.15	.23	.30	.27	.32	.19																																						
14	.49	.45	.40	.23	.24	.24	.21	.23	.33	.25	.31	.24	.05																																					
15	.40	.43	.35	.39	.34	.21	.20	.25	.27	.27	.32	.22	.13	.15																																				
16	.40	.43	.33	.37	.34	.19	.20	.24	.26	.28	.31	.22	.12	.14	.05																																			
17	.38	.44	.41	.37	.33	.16	.15	.24	.28	.34	.19	.22	.08	.15	.11	.10																																		
18	.70	.71	.83	.58	.58	.59	.52	.52	.73	.64	.58	.63	.54	.55	.54	.55	.56																																	
19	.70	.48	.77	.61	.59	.59	.56	.38	.67	.67	.59	.57	.36	.40	.59	.59	.55	.22																																
20	.51	.51	.62	.39	.41	.39	.37	.38	.52	.46	.57	.44	.37	.38	.37	.37	.37	.22	.05																															
21	.49	.45	.68	.56	.50	.42	.39	.37	.52	.61	.57	.48	.34	.38	.41	.41	.38	.21	.23	.02																														
22	.49	.45	.67	.57	.51	.41	.38	.38	.52	.63	.56	.48	.33	.37	.39	.39	.38	.19	.23	.00	.02																													
23	.65	.48	.77	.61	.65	.59	.60	.43	.70	.68	.66	.60	.41	.45	.60	.62	.59	.56	.59	.34	.53	.53																												
24	.55	.53	.76	.49	.54	.49	.50	.43	.70	.60	.54	.61	.45	.36	.50	.51	.49	.49	.43	.28	.40	.39	.29																											
25	.64	.47	.79	.64	.67	.58	.59	.50	.70	.71	.61	.62	.41	.42	.57	.58	.57	.58	.60	.36	.52	.52	.34	.21																										
26	.70	.44	.82	.55	.68	.61	.61	.43	.76	.73	.52	.63	.36	.38	.58	.60	.57	.49	.54	.30	.52	.51	.40	.19	.31																								
27	.67	.47	.78	.58	.65	.60	.60	.48	.70	.67	.63	.54	.42	.44	.59	.61	.56	.55	.55	.33	.50	.49	.34	.23	.26	.32																						
28	.59	.41	.75	.54	.64	.53	.53	.43	.66	.68	.51	.51	.35	.36	.51	.54	.50	.49	.52	.30	.46	.45	.35	.20	.28	.27	.21																					
29	.65	.43	.77	.52	.64	.56	.57	.40	.69	.71	.54	.53	.37	.37	.55	.58	.52	.49	.51	.30	.48	.47	.35	.22	.28	.31	.23	.13																				
30	.67	.45	.79	.55	.65	.58	.59	.39	.71	.72	.61	.55	.39	.42	.56	.59	.54	.52	.53	.30	.49	.49	.35	.20	.27	.29	.23	.21	.26																			
31	.58	.40	.73	.50	.60	.50	.51	.37	.63	.62	.51	.49	.32	.35	.51	.53	.47	.47	.47	.27	.44	.44	.26	.23	.29	.31	.25	.20	.20	.27																		
32	.59	.41	.77	.53	.65	.51	.51	.42	.67	.68	.54	.53	.36	.37	.53	.54	.51	.47	.50	.29	.44	.44	.34	.18	.24	.24	.15	.17	.17	.16	.16																	
33	.46	.40	.63	.40	.48	.39	.40	.41	.57	.54	.53	.49	.37	.36	.39	.40	.39	.48	.32	.27	.29	.29	.19	.16	.11	.10	.10	.09	.11	.08	.11	.05																
34	.57	.41	.71	.47	.57	.50	.50	.42	.66	.63	.52	.53	.36	.35	.47	.49	.48	.49	.48	.28	.42	.42	.32	.15	.21	.21	.14	.14	.14	.15	.14	.09	.04															
35	.62	.41	.73	.50	.60	.55	.55	.42	.67	.65	.54	.53	.37	.37	.52	.55	.51	.49	.52	.29	.48	.47	.32	.17	.24	.26	.17	.17	.15	.20	.15	.12	.05	.05														
36	.50	.42	.68	.46	.55	.43	.43	.42	.60	.59	.55	.50	.36	.38	.44	.46	.42	.47	.40	.29	.37	.37	.26	.18	.17	.18	.13	.12	.11	.13	.11	.08	.07	.06	.05													
37	.56	.43	.76	.51	.61	.49	.49	.42	.66	.66	.53	.51	.32	.38	.51	.53	.46	.51	.52	.31	.47	.47	.32	.22	.28	.29	.24	.13	.13	.24	.13	.15	.11	.11	.10	.08											
38	.57	.43	.75	.51	.60	.49	.50	.42	.65	.65	.53	.51	.32	.37	.50	.53	.45	.51	.51	.29	.46	.46	.33	.22	.26	.30	.22	.14	.14	.24	.14	.14	.10	.10	.09	.07	.03										
39	.61	.42	.73	.51	.60	.53	.54	.42	.67	.66	.51	.52	.37	.38	.52	.55	.50	.47	.50	.28	.46	.46	.32	.18	.23	.26	.19	.16	.18	.23	.17	.12	.06	.07	.05	.04	.10	.08									
40	.61	.42	.73	.51	.59	.53	.54	.43	.67	.64	.54	.52	.37	.38	.51	.53	.49	.49	.51	.29	.46	.46	.32	.18	.22	.26	.20	.17	.19	.23	.17	.11	.06	.07	.06	.05	.10	.09	.05								
41	.58	.40	.72	.48	.58	.50	.51	.36	.64	.64	.48	.49	.33	.34	.49	.52	.47	.42	.47	.23	.43	.43	.29	.16	.22	.24	.14	.16	.13	.17	.12	.10	.04	.05	.04	.03	.05	.04	.01	.01							
42	.58	.40	.72	.48	.59	.50	.51	.36	.65	.65	.49	.49	.34	.34	.50	.52	.47	.43	.48	.24	.44	.44	.30	.16	.22	.24	.15	.16	.13	.17	.12	.09	.04	.05	.04	.03	.05	.04	.01	.01	.00						
43	.59	.42	.71	.59	.62	.53	.54	.40	.62	.67	.55	.48	.37	.36	.51	.51	.50	.44	.51	.26	.44	.43	.45	.32	.45	.40	.37	.37	.35	.38	.32	.31	.20	.29	.33	.25	.34	.34	.32	.33	.30	.30					
44	.50	.40	.62	.51	.52	.44	.43	.39	.54	.53	.50	.48	.35	.33	.40	.41	.40	.47	.43	.27	.35	.35	.37	.29	.33	.32	.27	.25	.27	.29	.22	.24	.17	.23	.24	.24	.26	.26	.24	.24	.22	.22	.11				
45	.48	.40	.64	.52	.53	.40	.40	.40	.53	.55	.52	.46	.35	.35	.40	.40	.38	.46	.42	.27	.33	.33	.36	.32	.33	.33	.27	.26	.27	.29	.21	.23	.20	.25	.24	.23	.26	.25	.24	.25	.22	.22	.10	.05			
46	.47	.39	.62	.54	.52	.41	.41	.39	.53	.54	.49	.48	.35	.33	.38	.39	.37	.46	.49	.27	.35	.34	.41	.29	.39	.40	.36	.34	.37	.37	.30	.31	.17	.27	.33	.23	.34	.34	.32	.33	.31	.31	.17	.03	.04		
47	.54	.52	.74	.49	.57	.50	.49	.38	.65	.59	.50	.54	.50	.39	.50	.52	.49	.47	.44	.31	.43	.43	.41	.39	.45	.39	.42	.39	.38	.39	.35	.36	.38	.37	.38	.37	.38	.38	.38	.38	.34	.34	.38	.37	.36	.36	
48	.53	.50	.68	.47	.51	.49	.48	.39	.60	.52	.52	.55	.46	.36	.46	.46	.49	.46	.39	.30	.39	.38	.39	.33	.37	.31	.38	.33	.35	.36	.32	.32	.31	.30	.31	.33	.35	.34	.31	.32	.30	.30	.32	.29	.31	.28	.27
49	.59	.41	.73	.61	.57	.55	.54	.41	.64	.65	.53	.54	.37	.35	.54	.53	.54	.49	.52	.28	.47	.47	.47	.35	.41	.50	.41	.38	.41	.43	.37	.37	.25	.34	.39	.30	.41	.39	.38	.38	.37	.38	.39	.29	.29	.36	.24	.18
50	.49	.41	.64	.56	.52	.42	.41	.37	.54	.57	.54	.47	.36	.34	.41	.41	.39	.45	.50	.27	.36	.35	.46	.35	.45	.49	.42	.38	.40	.44	.37	.36	.25	.34	.38	.27	.40	.39	.39	.39	.36	.36	.32	.21	.20	.20	.22	.16	.22
51	.39	.39	.57	.43	.45	.34	.32	.35	.46	.49	.49	.36	.34	.35	.34	.34	.33	.45	.34	.26	.29	.30	.31	.35	.30	.33	.24	.23	.23	.25	.21	.22	.25	.23	.23	.21	.24	.22	.22	.23	.20	.21	.21	.19	.18	.19	.16	.18	.07	.05
52	.44	.40	.65	.57	.51	.38	.36	.39	.51	.55	.52	.45	.31	.34	.40	.39	.36	.46	.49	.27	.36	.34	.45	.34	.45	.47	.41	.36	.40	.43	.35	.35	.25	.34	.38	.26	.36	.35	.37	.38	.36	.36	.32	.20	.18	.19	.23	.17	.21	.06	.03

1, #Aquimarina_sp_RKVR24;2, #PS35R1_3; 3, #AH30R2_1; 4, #PS30R2_2; 5, #AH30R2_3; 6, #Corynebacterium_sp_Triatoma_infestans; 7, #Mycobacterium_abscessus_strain_DS27; 8, #AH35R2_6; 9, #PL40_4; 10, #AH30R1_3; 11, #AM35R2_4; 12, #AH40R1_3; 13, #Kytococcus_sp_RKEM_512A; 14, #AM30R2_8; 15, #Brachybacterium_rhamnosum_strain_VB212; 16, #Brachybacterium_sp_HBUM178917; 17, #Ornithinimicrobium_sp_VN123; 18, #PS35R2_1; 19, #PS30R2_1; 20, #Gamma_proteobacterium_M37; 21, #Alteromonadaceae_bacterium_GUDS1097; 22, #Microbulbifer_sp_2ta13; 23, #PL25R1_5; 24, #AH35R2_7; 25, #PL35R2_2; 26, #PS25R2_2; 27, #PL35R2_6; 28, #PS25R2_4; 29, #PS35R2_11; 30, #PL35R1_14; 31, #Paracoccus_sp_MBIC3024; 32, #PS25R1_9; 33, #AM30R1_3; 34, #PS35R1_2; 35, #PL30R1_1; 36, #Ruegeria_atlantica_strain_D7087; 37, #Rhodobacteraceae_bacterium_B7ZZ2008; 38, #Phaeobacter_caeruleus_strain_DSM_24564; 39, #Ruegeria_sp_F75200; 40, #Ruegeria_lacuscaerulensis_strain_F77045;41, #Ruegeria_sp_JZ10IS2; 42, #Ruegeria_sp_JZ10ML44; 43, #PS30R1_8; 44, #PS35R2_5; 45, #PS35R1_6; 46, #Alpha_proteobacterium_JE066; 47, #AM35R2_5; 48, #PL25R2_11; 49, #PL25R2_13; 50, #Erythrobacter_nanhaisediminis_strain_F75112; 51, #Erythrobacter_flavus_strain_N54; 52, #Erythrobacter_sp_OC8S.