

# Insights into the cultured bacterial fraction of corals

Sweet, Michael<sup>1\*#</sup>; Villela, Helena<sup>2\*</sup>; Keller-Costa, Tina<sup>3\*</sup>; Costa, Rodrigo<sup>3,4\*</sup>; Romano, Stefano<sup>5\*</sup>; Bourne, David G.<sup>6</sup>; Cárdenas, Anny<sup>7</sup>; Huggett, Megan J.<sup>8,9</sup>; Kerwin, Allison H.<sup>10</sup>; Kuek, Felicity<sup>11</sup>; Medina, Mónica<sup>11</sup>; Meyer, Julie L.<sup>12</sup>; Müller, Moritz<sup>13</sup>; Pollock, F. Joseph.<sup>11,14</sup>, Rappé, Michael S.<sup>15</sup>; Sere, Mathieu<sup>1</sup>; Sharp, Koty H.<sup>16</sup>; Voolstra, Christian R.<sup>7</sup>; Zaccardi, Nathan<sup>16</sup>, Ziegler, Maren<sup>17</sup>; Peixoto, Raquel<sup>2,18,19\*</sup>

<sup>1</sup>Aquatic Research Facility, Environmental Sustainability Research Centre, University of Derby, DE22 1GB, UK

<sup>2</sup>Federal University of Rio de Janeiro, Brazil

<sup>3</sup>Institute for Bioengineering and Biosciences (iBB), Instituto Superior Técnico (IST), University of Lisbon, 1049-001 Lisbon, Portugal

<sup>4</sup>Department of Energy – Joint Genome Institute and Lawrence Berkeley National Laboratory, Berkeley, California 94720, USA

<sup>5</sup>Gut Microbes and Health, Quadram Institute Bioscience, Norwich, NR4 7UQ, UK

<sup>6</sup>College of Science and Engineering, James Cook University and Australian Institute of Marine Science, Townsville, 4810, Australia

<sup>7</sup>Department of Biology, University of Konstanz, Konstanz, Germany

<sup>8</sup>School of Environmental and Life Sciences, The University of Newcastle, 10 Chittaway Rd, Ourimbah 2258 NSW Australia

<sup>9</sup>Centre for Marine Ecosystems Research, Edith Cowan University, 270 Joondalup Dr, Joondalup 6027 WA Australia

<sup>10</sup>Department of Biology, McDaniel College, Westminster, MD, 21157, USA

<sup>11</sup>Department of Biology, Pennsylvania State University, University Park, PA 16802

<sup>12</sup>Soil and Water Sciences Department, Genetics Institute, University of Florida, Gainesville, FL, USA

<sup>13</sup>Faculty of Engineering, Computing and Science, Swinburne University of Technology Sarawak Campus, 93350 Kuching, Sarawak, Malaysia.

<sup>14</sup>Hawaii and Palmyra Programs, The Nature Conservancy, 923 Nuʻuanu Avenue, Honolulu, HI 96817

<sup>15</sup>Hawaii Institute of Marine Biology, University of Hawaii, P.O. Box 1346, Kaneohe, HI, 96744, USA

<sup>16</sup>Department of Biology and Marine Biology, Roger Williams University, Bristol, RI, 02809, USA

<sup>17</sup>Department of Animal Ecology and Systematics, Justus Liebig University Giessen, Giessen, Germany

<sup>18</sup>IMAM-AquaRio – Rio de Janeiro Aquarium Research Center, Rio de Janeiro, Brazil.

<sup>19</sup>Genome Center, University of California Davis, USA.

\*Authors contributed equally

#corresponding author: [m.sweet@derby.ac.uk](mailto:m.sweet@derby.ac.uk)

## Abstract

Bacteria associated with coral hosts are diverse and abundant, with recent studies suggesting involvement of these symbionts in host resilience to anthropogenic stress. Despite the putative importance of bacteria, the work dedicated to culturing coral-associated bacteria has received little attention. Combining published and unpublished data, here we report a comprehensive overview of the diversity and function of culturable, coral-associated bacteria. A total of 3055 isolates from 52 studies were considered by our meta-survey. Of these, 1045 had full length 16S rRNA gene sequences, spanning 138 formally described and 12 putatively novel bacterial genera across the Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria phyla. We performed comparative genomic analysis using the available genomes of 74 strains and identified potential signatures of beneficial bacterial-coral symbioses among them. Our analysis revealed >400 biosynthetic gene clusters that underlie the biosynthesis of antioxidant, antimicrobial, cytotoxic, and other secondary metabolites. Moreover, we uncovered genomic features - not previously described for coral-bacterial symbioses - involved in host colonization and host-symbiont recognition, antiviral defence mechanisms, and/or integrated metabolic interactions, which we suggest as novel targets for the screening of coral probiotics. Our results highlight the importance of bacterial cultures to elucidate coral holobiont functioning, and guide the selection of probiotic candidates to promote coral resilience and improve reef restoration efforts.

KEY WORDS: symbiosis, holobiont, metaorganism, cultured microorganisms, coral, probiotics, beneficial microbes

## Introduction

In recent years, the concept of the metaorganism or ‘holobiont’ has become a cornerstone of biology <sup>1</sup>, although the view of the holobiont as the unit of selection is a matter of current debate <sup>2</sup>. Notwithstanding, the term metaorganism has gained momentum in modern studies of complex host-microbe interactions, as it defines the association formed by a host organism and its microbiome <sup>2,3,4</sup>. Scleractinian corals are an excellent example of such an association, as they build reefs through close symbiotic interactions between the host modular animal, its endosymbiotic dinoflagellates (Symbiodiniaceae) and an array of other microbial partners

including bacteria, archaea and fungi <sup>1</sup>. The bacterial taxa associated with corals can vary between coral species and geographical origin, though often there are patterns in the community structure that link microbial and coral taxa <sup>5,6</sup>. The majority of recent studies exploring the importance of coral-associated microbes have focused on the use of cultivation-independent approaches, based on 16S rRNA gene amplicon sequencing <sup>7</sup> and, more recently, shotgun metagenomics <sup>8</sup>. Such methods are central in identifying what bacteria are associated with corals and how their metabolic and functional potential contribute to holobiont health and response to environmental conditions <sup>7,9 10</sup>. However, the bacterial metabolic pathways that interact with the host and respond to environmental changes are often best understood using culture-based approaches <sup>11</sup>. This is particularly relevant because metagenomic information gives insights into potential functional traits and other cellular traits only, and often environmental changes have pleiotropic effects on holobiont physiology that are impossible to grasp using metagenomics alone <sup>12,13,14,15</sup>.

Inherently, culture-based approaches retrieve only a small fraction of the total bacterial diversity within any given environment, a phenomenon known as the “great plate anomaly” <sup>16,17,18</sup>. Often however, it is not a case of being ‘unculturable’, but of not yet knowing the range of conditions needed to culture these microorganisms. Cultivating host-associated microorganisms can be challenging, as their nutrient requirements and cross-feeding networks are often unknown <sup>19</sup>. In addition, many environmental microorganisms grow very slowly (in contrast to clinical isolates), and are not adapted or capable of growing on commonly used nutrient-rich media <sup>20,21</sup>. To counter this, at least to some degree, recent studies have implemented novel and alternative culture-based methods to retrieve a higher proportion of the bacterial diversity in any given sample <sup>19,22</sup>, and these approaches have also been applied to corals <sup>23,24,25</sup>.

Tissue compartmentalization and organismal complexity are thought to underlie high bacterial diversity in corals <sup>26,27,28</sup>. The diverse coral bacteriome plays an integral role in the balance between health and disease of the coral holobiont <sup>29</sup> and represents a valuable source of biotechnological products <sup>30</sup>. <sup>31</sup> was perhaps the first to actively isolate bacteria from coral, recovering strains from the skeletal regions of *Porites lobata* followed by <sup>32</sup> who reported on bacteria isolated from mucus of *Porites astreoides* and two soft coral species. Microbial mediated diseases have also been well documented as driving declines in reef health,

especially throughout the Caribbean for example <sup>33</sup>. This has fostered a great interest in understanding coral disease causative agents, stimulating cultivation efforts of coral-associated bacteria <sup>34,35,36</sup>. For example, <sup>37</sup> isolated a bacterium that caused bleaching of the coral *Oculina patagonica*, and many subsequent studies have implicated vibrios in coral disease causation <sup>38,39,40</sup>. Many of these focused on targeted isolation and conducted reinfection studies to satisfy Koch's postulates (reviewed in <sup>41</sup>).

Similarly, growing evidence underlines the key role compounds produced by bacteria have on host health<sup>42,43,29,44,45</sup>. For instance,<sup>46</sup> was among the first to demonstrate that mucus-associated bacteria from healthy colonies inhibit the growth of potential pathogens, like the vibrios mentioned above. Subsequent studies revealed high antimicrobial activity among culturable coral-associated bacteria, with up to 25% of the isolates producing antimicrobial compounds <sup>47</sup>. <sup>48</sup> showed a strong link between observed antibiotic activity in well diffusion assays and existence of polyketide synthase (PKS) and/or non-ribosomal peptide synthetase (NRPS) genes in the bacterial isolates. More recently, <sup>13</sup> identified the antimicrobial compound tropodithietic acid (TDA) to be produced by the coral-associated bacterium *Pseudovibrio* sp.. In fact, interestingly, *Pseudovibrio* species harbour several biosynthetic gene clusters for the synthesis of bioactive compounds <sup>49,50</sup>.

These studies reinforce the notion that the isolation of bacteria from corals is a vital part of coral science. They are invaluable for assessing the virulence of potential pathogens, and for applying classical clinical approaches to elucidate disease aetiology <sup>51</sup>. Beneficial traits that bacteria may provide to coral holobiont functioning can also only be proven using pure bacterial cultures <sup>14,8</sup>. Perhaps most importantly, bacteria isolated from corals can be used as probiotics to facilitate host health <sup>52,53</sup>. Such approaches have been proposed to promote coral resilience in the face of environmental stress. For example, <sup>44</sup> showed that application of these 'beneficial microorganisms for corals' (or BMCs) increases the resilience of the coral to temperature stress and pathogen challenge.

Here, we sought to centralize and curate the current cultured fraction of coral bacteria by combining published data with unpublished collections from around the world. We explore relationships between the bacteria acquired and the host origin and the media utilized for growth. A total of 74 genomes of cultured coral bacteria, 36 of which are available in public

databases and 38 of which are presented in this study for the first time, were investigated to infer potential genetic signatures that may facilitate a host-associated lifestyle. Alternative ways and improvements for the isolation of bacterial groups not yet recovered from corals (including targeting specific obligate symbionts) are also discussed. This study provides the most comprehensive synthesis of the cultured bacterial fraction of the coral holobiont to date.

## **Methodology**

### **Literature search and data curation**

The National Center for Biotechnology Information (NCBI) was searched for publicly available 16S rRNA gene sequences of cultured coral-associated bacteria. This was supplemented with culture collections from laboratories around the world through a public invitation to researchers working in this field. The initial invite started through social media (Twitter), then developed by word of mouth. In total, we were able to obtain bacterial isolates originating from 84 coral species (representing tropical, temperate, and cold-water habitats), and representatives from all major oceans (**Figure 1**; supplementary material, Supplementary **Tables 1**).

### **Phylogenetic analysis and tree generation**

In total, we were able to collate 3055 individual isolates which had a part of the 16S rRNA gene sequenced (**See Supplementary Table 1 for details**). Sequences with less than 500 base pairs (bp), longer than 1600 bp, or containing more than 1 ambiguity were removed. Screen-seqs (mothur version.1.42.0) was used to remove poorly aligned sequences and filter-seqs was used to remove positions without sequence information <sup>54</sup>. This left 1045 isolates with near full-length 16S rRNA gene sequences. These were then aligned using the SILVA 138.1 database as a reference <sup>55</sup>, and the clear-cut command was used within mothur to generate a phylogenetic tree. The tree was constructed using the Maximum Likelihood method based on the Tamura-Nei model. A phylogenetic tree of coral hosts was also generated using the Taxonomy Common Tree tool of NCBI <sup>56</sup>. Species names were added manually to create a tree file. Tree features were optimized using iTOL v4 <sup>57</sup>.

## **Taxonomic composition of bacterial isolates by medium**

Bacterial strains listed in **Supplementary Table 1** were sorted by isolation medium and subsequently grouped at phylum, order, and genus levels according to the current SILVA (138.1) taxonomy<sup>55</sup>. Stacked column graphs, showing relative abundances of the cultivated taxa were created thereafter. At the genus level, all groups representing less than 1% of the total pool in each medium were included in a group labelled “others”.

## **Genome analysis**

The integrated Microbial Genomes and Microbiomes database (IMG; <https://img.jgi.doe.gov/>) from the Joint Genome Institute (DOE-JGI), and the assembly database from NCBI (<https://www.ncbi.nlm.nih.gov/assembly>) were searched for publicly available genomes from cultured coral bacteria in February 2020. Thirty-six bacterial genome assemblies (21 from scleractinian coral and 15 from octocoral associates) were downloaded from NCBI and included in this analysis. A further 38 bacterial genomes from scleractinian corals were uploaded as part of this manuscript (Project Accession Nos. GS0145871, and PRJNA343499). The annotation of general genomic features such as genome size, GC-content, and number of coding sequences (CDSs) was performed for all 74 genomes with the RAST server<sup>58</sup> (**see Supplementary Table 2**). Protein families (Pfam) were predicted with the online-server WebMGA (default settings)<sup>59</sup> and using amino-acid sequence files obtained from RAST. The resulting individual Pfam annotation files were then joined using a customized script in R and the resulting count tables were Hellinger-transformed for multivariate analyses. Dissimilarity between genomes based on the Pfam profiles were then calculated using the Bray-Curtis index and data clustered using a principal coordinate analysis (PCoA) and plotted in Eigenvalue scale (i.e. scaling of each axis using the square root of the Eigenvalue) using PAST v3.25<sup>60</sup>. PERMANOVAs (permutational multivariate analysis of variance) were performed with 999 permutations to test for overall differences in the Bray-Curtis dissimilarity matrix between Pfam profiles of bacterial genomes from different taxonomic classes. Five groups (classes) were used: Alphaproteobacteria, Gammaproteobacteria, Actinobacteria, Cytophagia, and Flavobacteriia. A separate PERMANOVA analysis of Bray-Curtis dissimilarities calculated for the 11 available *Vibrio* genomes was then performed in order to highlight differences between strains identified as

potentially pathogenic (n = 5, group 1) and those apparently non-pathogenic (n = 6, group 2) (identification of pathogenicity from available literature – see references). Finally, AntiSMASH version 5.0<sup>61</sup> was used with default parameters to identify biosynthetic gene clusters (BGCs) in all genomes.

## **Results**

### **Phylogenetic analysis of culturable coral-associated bacteria**

Published and unpublished datasets were interrogated, identifying 3055 cultured coral-associated bacteria, for which 1045 high-quality full-length 16S rRNA gene sequences were available (**Figure 2a**). This collection demonstrates that many bacterial genera (138) can be cultured from corals. While most isolates belong to the phylum Proteobacteria (72% of those cultured), strains from Firmicutes (14%), Actinobacteria (10%), and Bacteroidetes (5%) were also recovered. The genera *Ruegeria*, *Photobacterium*, *Pseudomonas*, *Pseudoalteromonas*, *Vibrio*, *Pseudovibrio*, and *Alteromonas* were commonly isolated across studies (**Figure 2 and 3**; see **Supplementary Tables 1, 3 and 4**). Of 43 genera identified as likely beneficial microbes (proposed in current literature see references in **Supplementary Table 4**), 25 (58%) have been cultured and are represented in this collection (**Supplementary Table 4**). Most of the isolates that have been cultured from diseased corals belong to the phylum Proteobacteria, specifically to the family Vibrionaceae. However, it should be noted that many of the studies reporting these focused on a targeted approach to isolate these bacteria. Among the isolates from the Proteobacteria phylum, 25.5% were associated with diseased coral colonies, as were 7.4% of the isolates belonging to the phylum Bacteroidetes. Firmicutes and Actinobacteria had the lowest percentages, with 5.0% and 0.7%, respectively. Although the majority of the isolates were matched with other representatives on GenBank, 12 were highly divergent with low identify to known isolates suggesting that they may be novel genera.

### **Taxonomic composition of bacterial isolates by culture medium**

The taxonomic patterns of the cultured bacterial strains at phylum, order, and genus level, vary according to the type of medium used to isolate them (**Figure 4**). Marine agar (MA) (including its diluted versions) was the most commonly utilised medium across studies, and produced 715 unique isolates collectively. Bacterial isolates belonging to the families

Vibrionaceae, Alteromonadaceae, Pseudoalteromonadaceae, Rhodobacteraceae, Flavobacteriaceae, and Micrococcaceae could all be isolated from MA from a diverse set of coral species. The next most productive non selective medium, glycerol artificial seawater agar (GASWA), produced 572 isolates, while a variety of ‘custom’ media from different laboratories produced 523 isolates. Interestingly, this latter collection of media i.e. the custom variants (along with blood agar specifically), favoured the retrieval of Firmicutes and Actinobacteria species at the expense of Proteobacteria representatives. In contrast, media commonly deployed to sample a wider bacterial diversity, such as marine agar, favoured the growth of several Proteobacteria species, which in turn is usually affiliated with diverse clades within the Alphaproteobacteria and Gammaproteobacteria classes (**Figure 2 and 4**). Curiously, use of Thiosulfate-citrate-bile salts-sucrose medium (TCBS) supported the growth of manifold bacterial lineages across the four phyla documented in this study, despite its presumed selectivity for *Vibrio* species.

As mentioned, the majority of the isolates (72%) belonged to the Proteobacteria. Indeed, members of this phylum could be retrieved from nearly all cultivation media and conditions examined in our survey – in higher or lower numbers, according to the design and scope of the study (**Figure 2**). Bacteria belonging to the phyla Firmicutes, Bacteroidetes, and Actinobacteria, also appeared to be cultured on most media, though in lower numbers (**Figure 4a**). Orange Serum Agar seemed to be selective for Actinobacteria (**Supplementary Table 1**). The media MA, R2A, and minimal basal agar shared a very similar pattern at the order level, with all yielding similar proportions of members from the orders Vibrionales, Rhodobacterales, Pseudomonadales, Flavobacteriales and Actinomycetales (**Fig. 4**). Likewise, LB, blood agar, and the ‘custom’ media shared similar proportion patterns, which included the orders Vibrionales, Pseudomonadales, Bacillales, Alteromonadales, and Actinomycetales (**Figure 4b**). At the genus level, no immediate patterns seemed to be shared among the media (**Figure 4c**). The highest number of unique isolates identified to genus level was obtained from MA (115), followed by custom media (55), minimal basal media (48), and GASWA (47) (**Supplementary Table 1**). However, when dividing the number of different genera by the total number of isolates in each medium, the normalized ratios show that nutrient agar (0.64), followed by DMSP-enriched media (0.54) and R2A (0.4), supported the growth of higher bacterial diversity. Conversely, lowest bacterial diversities were found on TCBS (0.04), Nfb

(0.04), and GASWA (0.08). The normalized ratios for each medium (considering all the isolates analysed here) can be found in **Supplementary Table 1**.

## **Functional genomics of coral bacterial isolates**

A total of 74 cultured coral associated bacteria had full or draft genomes available (36 genomes were accessible as of February 2020, with a further 38 genomes now available from this study (**Supplementary Table 2**). The genome sizes ranged from 2.71 Mb in *Erythrobacter* sp. A06\_0 (associated with the scleractinian coral *Acropora humilis*) with only 2669 coding sequences (CDSs), to 7.28 Mb in *Labrenzia alba* EL143 (associated with the octocoral *Eunicella labiata*) with 4551 CDSs (**Supplementary Table 2**). The mean and median genome size was 4.77 Mb and 4.71 Mb, respectively. The average GC-content of these genomes was 52.99%, with the lowest GC-content (32.9%) found in *Aquimarina megaterium* strain EL33 (isolated from *E. labiata*), and the highest GC-content (71.4%) found in *Janibacter corallicola* strain NBRC 107790 (from *Acropora gemmifera*).

Multivariate analysis, based on protein family (Pfam) profiles (**Figure 5a**), unsurprisingly showed that the genomes grouped mostly according to their (class-level) taxonomic affiliations (PERMANOVA,  $F = 11.55$ ,  $P = 0.0001$ ). Exceptions were the two Actinobacteria and the two Bacteroidetes genomes, which clustered with four Alphaproteobacteria genomes of the order Sphingomonadales and Caulobacterales and the *Luteimonas* sp. JM171 (Gammaproteobacteria) genome, respectively. However, this is likely a reflection of the very low number of genomes available from coral-associated Actinobacteria and Bacteroidetes rather than a significant functional overlap between the two phyla. Interestingly, a PERMANOVA analysis performed on the Pfam profiles of the Vibrionales genomes revealed that the five *Vibrio* genomes from known pathogens were significantly different from all non-pathogenic Vibrionaceae strains ( $P = 0.0006$ ,  $df = 1$ ,  $F = 1.829$ ).

Functions that potentially have a role in host-microbial interactions, such as proteins containing Eukaryotic-like domains involved in host-symbiont recognition<sup>62,63</sup>, secretion systems important for host colonization, and biosynthetic gene clusters encoding for secondary metabolites were investigated across the isolates (**Figure 5b**). The *Endozoicomonas* strains G2\_1, G2\_2 and Acr-14 had the highest number of ankyrin repeats (> 789), and high

numbers of WD40 repeats (between 37-116). All Alteromonadales strains (including the *Pseudoalteromonas* BMCs), had high numbers of tetratricopeptide (>250) and WD40 (29-142) repeats. The strain with the overall highest number of Eukaryotic-like repeat protein related entries (1367 repeats) was *Endozoicomonas* sp. strain G2\_01 from *Acropora cytherea*, closely followed by the octocoral associate *Aquimarina megaterium* EL33 (class Flavobacteria) (1208 repeats). By contrast, ankyrin repeats were absent or only present in low numbers in all the *Vibrio* strains. *Endozoicomonas montiporae* strain EL-33 displayed the highest number of domains related to antiviral defence mechanisms, such as CRISPR proteins and endonucleases, which are known to be enriched in the microbiomes of marine sponges<sup>63,64</sup>. Further, 49 out of the 74 genomes assessed, harboured the TauD (PF02668) gene. TauD is involved in the degradation of host-derived taurine into sulfide which is then assimilated into microbial biomass<sup>65,66,67</sup>. An elevated number of TauD encoding CDSs was found in the two BMC strains *Cobetia marina* BMC6 and *Halomonas tateanensis* BMC7, both isolated from *Pocillopora damicornis*. Further, several isolates ( $N = 11$ ) of the Rhodobacteraceae family (Alphaproteobacteria) contained CDSs involved in dimethylsulfoniopropionate (DMSP) degradation, potentially contributing to sulphur cycling in corals.

Among secretion systems, type II (T2SS), III (T3SS), IV (T4SS), and VI (T6SS), known to be involved in host colonization e.g.<sup>68</sup>, horizontal gene transfer e.g.<sup>69</sup>, or interbacterial antagonism and/or virulence e.g.<sup>70</sup>, dominated the genomes of coral-associated bacteria. We found a high number of entries related to T2SS in the Gammaproteobacteria associates, particularly in the *Endozoicomonas* and *Vibrio* genomes (see<sup>71</sup> for roles of the T2SS in symbiosis and pathogenicity). The Vibrionales genomes were further characterised by an elevated number of T6SS-related Pfam domains, whereby the five pathogenic *Vibrio* strains encoded a significantly higher number of T6SS domains (mean=27 T6SS domains in CDSs) than the six non-pathogenic *Vibrio* strains (mean=10 T6SS domains in CDSs; Man-Whitney U-test,  $p = 0.0126$ ).

We also assessed the secondary metabolite coding potential in the 74 genomes. AntiSMASH v.5.0 detected a total of 416 biosynthetic gene clusters (BGCs) across all genomes, whereby the number of BGCs varied substantially between strains, from zero BGCs in *Endozoicomonas montiporae* CL-33 to 12 BGCs in *Pseudoalteromonas luteoviolacea* HI1 (**Figure 6**). Bacteriocin clusters ( $N = 75$ ), found in 81% of the strains, were the most frequently detected BGCs,

followed by homoserine lactone ( $N = 62$ ; in 43% of strains), non-ribosomal peptide synthetase (NRPS;  $N = 59$ ; in 51% of strains), beta-lactone ( $N = 46$ ; in 53% of strains), terpene ( $N = 34$ ; in 38% of strains), ectoine ( $N = 28$ ; in 35% of strains), and siderophore ( $N = 25$ ; in 28% of strains) clusters. The relatively large group of coral-associated Rhodobacteraceae genomes analysed in this study presented a consistently rich BGC profile, characterised by the presence of bacteriocin, homoserine lactone, and NRPS-T1PKS clusters, while siderophore clusters were typically absent in this group. Siderophores were typically found in the *Vibrio* genomes of this study as well as in three of the four *Endozoicomonas* genomes. Characteristic for all *Pseudoalteromonas* genomes, including the BMC strains, was the presence of aryl polyene clusters, a compound class functionally related to antioxidative carotenoids <sup>72</sup>. The absence of known BGC in the genome of *E. montiporae* CL-33 is an unusual outcome, as for example the closely related strains in the Oceanospirillales order usually display > 4 BGCs (**Figure 6**). The *E. montiporae* CL33 genome is complete (100% completeness, 0.9% contamination, 95.5% quality; 1 contig); hence, low assembly quality - which sometimes compromises the identification of large BGCs - does not explain the lack of BGCs in this genome.

## Discussion

Here we show that many coral-associated bacteria ( $n = 3055$ ), can be isolated using a variety of medium and culture conditions. 138 of these isolates (recruited from 52 studies) have been formally described and at least 12 are putatively novel bacteria genera. It is promising that such extensive phylogenetic diversity can be captured from a limited number of culture media employed in the examined studies. Additional diversity is therefore likely to be captured through the design of alternative cultivation procedures that may improve our capacity to "cultivate the as-yet-uncultured". Testimony to this is the observation that most of the strains assigned to the Firmicutes phylum in our metanalysis were obtained almost exclusively from the various "custom media" utilised by different labs and blood agar alone, illustrating how diversification in cultivation design can widen the phylogenetic spectrum of the organisms domesticated in such endeavours. In this regard, we anticipate that broader phylogenetic diversity will be gained within the culturable fraction if gradients in aerophilic conditions, temperature, and other physicochemical parameters are attempted along with innovative, less invasive techniques to extract microbial cells from the host matrix. Intriguingly, the richness of bacterial phyla uncovered in this study corresponds to the phyla more often

reported to dominate bacterial communities in corals by cultivation-independent studies<sup>9</sup>, namely Proteobacteria, Bacteroidetes, Actinobacteria, and Firmicutes, yet how diversity at lower taxonomic ranks within each phylum is captured remains to be determined. Another exciting challenge ahead of us, is the unveiling of host-microbe and microbe-microbe molecular interdependence networks (e.g. cross-kingdom signalling and cross-feeding cascades). Such knowledge would ultimately enable laboratory cultivation of so-far “unculturable”, coral-specific or enriched lineages. Microbial-host and microbe-microbe interactions rely on several functions that are often found in host-associated and free-living bacteria<sup>73,74,49,75</sup>. Hence, having pure cultures of coral-associated bacteria can help to identify the genomic features that could underpin the interaction with the host and its microbiome and lead to their experimental validation.

Although one of the initial aims of this study was to ascertain the percentage of culturable bacteria from any given coral species, it was deemed too speculative to report the findings due to variation in culture effort across the various studies. Indeed, this highlights the paucity of studies dedicated to determine exactly this, and there is an urgent need for such mechanistic projects deploying multiple culture media and conditions to comprehensively sample bacterial associates from a single or few host species to be undertaken (see<sup>76,77,25</sup> for examples of such studies on sponges and corals, respectively). Collectively, studies designed as such, hold promise in illuminating our view of the cultivability of coral bacterial communities in a straightforward manner, and in solidly delineating the “cultivability gap” that is yet to be bridged in future experiments.

That said, having a catalogue of cultures as presented here (and one which will hopefully be ever-expanding) means we are, from now on, in a position to increase our understanding of host-symbiotic relationships. The ability to describe, understand, and culture specific symbionts from any given organism (like corals) also opens up the potential to utilise them as pro-biotics to restore degraded habitats<sup>44,52</sup>. In addition, such a resource increases the possibility of identifying novel compounds of biotechnological interest<sup>78</sup>. This seems particularly relevant in the case of coral-microbe symbioses, which are known to rank as one of the most prolific sources of bioactive molecules in the oceans<sup>79</sup>.

A search in public databases (NCBI) found that, despite the 1045 cultured coral-associated bacterial sequences with full-length 16S rRNA gene sequences, only 36 had genomes available as of February 2020. Clearly, a systematic effort to disclose the genomic features of coral-associated bacteria is needed in order to better understand the holobiont ecology and identify potentially beneficial microbes. As part of this study, we were able to add a further 38 to this tally (**Supplementary Table 2**). Even with this addition, the number of publicly available coral-associated bacterial genomes remains scant and it is recognised that to more fully understand the roles of the cultivable fraction of coral bacteria, a thorough characterization of the species kept in culture, including genome sequencing, needs to be fostered alongside experimental biology and manipulative approaches. Moreover, a large collection of coral-associated genomes could also help to identify specific traits that are needed to thrive in the various niches within the hosts or point to those bacteria which offer a specific benefit to their host.

All of the available genomes were screened for an array of functions potentially important in establishing and maintaining interactions between bacterial symbionts and their marine invertebrate hosts. Overall, the *Endozoicomonas* and *Pseudoalteromonas* strains displayed high numbers of eukaryotic-like protein-encoding genes essential for host-symbiont recognition in well-studied systems such as marine sponges<sup>80,81,63</sup>. The strain with the second highest number of eukaryotic-like repeat protein related entries (1208 CDSs, after *Endozoicomonas* sp. G2\_1 with 1367 CDSs), was the octocoral-associate *Aquimarina* sp. EL33 (class Flavobacteria). In the current culture collection, 15 additional *Aquimarina* isolates are reported, from the scleractinian corals *Porites lutea*, *Pocillopora acuta*, *Stylophora pistillata*, *Acropora millepora*, *A. tenuis* and the octocoral *E. labiata*. Retrieving the genomes from these candidates will allow us to further explore these emerging patterns in greater detail. For example, a recent comparative genomics survey of host-associated and free-living *Aquimarina* species revealed complex secondary metabolite biosynthesis and polycarbohydrate degradation capacities<sup>82</sup>, but further investigation into their mechanisms of interactions with corals is warranted.

Only eight *Endozoicomonas* isolates (five of them type species) have so far been cultured (according to our collated information). These are from the octocorals *Eunicea fusca* and *Plexaura* sp. and the scleractinian corals *Montipora aequituberculata*, *Acropora cytherea*, *A.*

*hemprichii* and *Acropora*. sp. To date, only four of these (two from this study), have had their genomes sequenced (all from scleractinian corals)<sup>14,83</sup>. This is surprising given that numerous studies found that this genus is highly abundant in the healthy coral holobiont (e.g. reviewed in<sup>29,84</sup>. Future cultivation efforts should therefore be directed towards the Endozoicomonadaceae family, in order to increase the representation of their taxonomic and functional diversity in culture collections. In this regard, this study finds evidence that supplementing culture media with DMSP is an approach worth investing in future attempts to cultivate coral-associated *Endozoicomonas*. The metabolic data obtained from the comparative analysis of these four strains can be used, for example, to drive the selection of specific nutrients and conditions required to culture this particular genus of coral symbionts. Furthermore, there are 55 cultured *Pseudoalteromonas* strains in our collection which should also be explored regarding their symbiotic properties and their functional gene content (only 6 genomes currently available). Similar to *Endozoicomonas*, *Pseudoalteromonas* species are also frequent members of coral-associated microbiomes<sup>29</sup>. A number of *Pseudoalteromonas* have been shown to harbour high antimicrobial activity and many of these bacteria are isolated from coral mucus, lending support to the protective role the surface mucus layer has for the host and its importance in the coral holobiont's defense - against gram-positive coral pathogens in particular<sup>85</sup>. Indeed, five of the six *Pseudoalteromonas* (where genomes are available), were shown to be effective BMCs when corals were challenged with the coral pathogen *Vibrio coralliilyticus*<sup>44</sup>.

Having genomes available from the potential pathogens also allows for greater insight into coral biology, especially when interested in ascertaining pathogenicity-related traits<sup>86,87</sup>. For example, from the 11 *Vibrio* species where genomic data was available, we were able to show functional separation (based on Pfam profiles) of known pathogenic and non-pathogenic strains. This was further accompanied by a significantly higher abundance of CDSs encoding for the Type VI secretion system, important for virulence in the pathogenic strains<sup>70</sup>. Prevalence of siderophore-encoding genes was also noted in the Vibrionaceae strains, suggesting that these bacteria likely gain competitive advantages through efficient and extensive iron acquisition, which is a trait often seen in opportunistic and pathogenic bacteria<sup>88,89</sup>. Hypothetically, the selection of beneficial microbes that are also good siderophore producers could add to the biological control of these pathogens. Indeed, two proposed BMC

strains *Cobetia marina* BMC6 and *Halomonas taenensis* BMC7, harbour such siderophore clusters on their genomes and so did three of the four *Endozoicomonas* strains. However, the five *Pseudoalteromonas* BMC strains and the *Endozoicomonas montiporae* CL-33, had contrasting low numbers of BGCs, possibly indicating a reduced investment into secondary metabolism. Indeed, the low number of BGCs in these *Pseudoalteromonas* strains is in contrast to the established prevalence of biologically active compounds in many marine host-associated *Pseudoalteromonas* strains<sup>90</sup>. In part, this may reflect a limitation of the software utilised to detect genes for all secondary metabolites, as genes for common metabolites (such as for the production of the antibiotic marinocin and those that produce tetrabromopyrrole coral larval settlement cues by *Pseudoalteromonas*<sup>91,92</sup>), were not picked up. These bioinformatic limitations emphasize the importance of having bacterial cultures for the elucidation of the chemical ecology underpinning coral holobiont functioning.

Broader functional traits can also be ascertained from looking at the complete picture of isolates with annotated genomes. For example, 66% (49 out of 74) harboured the TauD gene, which is involved in taurine utilization<sup>93</sup>. Two proposed BMCs, the *Cobetia marina* BMC7 and *Halomonas taenensis* BMC7, revealed the highest copy number of TauD CDSs (seven and eight, respectively), while others range between one and five TauD copies. Taurine is an organo-sulphur compound widely present in animal tissues, and recent research has shown that obligate symbionts of sponges have enriched copies of taurine catabolism genes and taurine transporters in comparison with free-living bacteria<sup>63,67,66</sup>. The widespread capability of the isolates studied here to potentially utilize host-derived taurine, could guide the formulation of novel, taurine-containing cultivation media in the attempt to captivate coral symbionts, particularly from the important, yet underrepresented order Oceanospirillales (TauD was consistently present in all Oceanospirillales genomes ( $N=8$ ) analysed here). The ubiquitous occurrence of bacteriocin clusters among the genomes is another example of broad scale trends which we have identified in our genome meta-analysis. These may confer the specific culturable symbionts with particular competitive capacities towards closely related taxa in highly dense microbiomes, as is commonly identified across corals and sponges<sup>94,95</sup>. Moreover, the widespread presence of NRPS and beta-lactone clusters hint towards broad-spectrum antimicrobial and cytotoxic capabilities in multiple associates. It also corroborates the hypothesis that these marine metaorganisms are promising sources of novel

bioactive compounds, representing targets for bioprospection<sup>79</sup>. Many strains also possess homoserine lactone encoding BGCs indicative for sophisticated, cell-density dependent chemical communication mechanisms. Antioxidant activities are likely conferred by the presence of aryl polyene BGCs in the genomes<sup>96</sup>. These pigment type compounds, functionally related to carotenoids, characterised most of the proposed BMC strains. Furthermore, several coral-associated bacteria of different taxonomic origins are seemingly well equipped to handle osmotic stress as revealed by the occurrence of ectoine and N-acetylglutaminyglutamine amide (NAGGN) encoding genes. Therefore, there is a need to continue the effort in culturing coral-associated bacteria to explore new biosynthetic potentials, both for bioprospecting purposes and for better understanding the chemical ecology of the metaorganism.

Identifying likely candidates for symbiosis is one challenge; but, once these are confirmed and characterised, the need to understand how the host establishes symbiosis and retains the relationship will also be critical. However, this is a two-way street. Current research in sponges has revealed that bacteria expressing the ankyrin genes avoid phagocytosis by sponge amoebocytes, thus becoming residents of the sponge microbiome by evading the host's immune system<sup>64</sup>. The evolutionary forces shaping the symbiosis are even trickier here, as bacteriophages encode for ankyrin biosynthesis in their genomes and might transfer this information across different community members<sup>64</sup>. Further, as ankyrin repeats are enriched in the microbial metagenomes of healthy corals<sup>8</sup>, a similar pattern of symbiosis establishment would be expected for corals.

To conclude, here we have highlighted that diverse coral-associated bacteria are already cultured, although these are often scattered across collections and rarely collated into one easily accessible location. Further, only a few of these have had their genomes sequenced. In spite of the lack of genomes we were able to identify a number of genetic features that seem to be enriched in these coral bacterial associates. These include the production of broad-spectrum antimicrobial, antioxidant, and cytotoxic capabilities, high abundance of ankyrin repeat entries, tetratricopeptide, and WD40 repeats, and taurine degradation genes. We have also observed a reduced investment into secondary metabolism, as a feature, in a number of coral bacterial associates. That said, this can only be quantitatively assessed in a robust manner if we could compare metagenome profiles from corals vs. other environments,

such as sediments and seawater in a comprehensive fashion (several samples with replication etc). Such metagenome-based analyses should be complemented by (large scale) marker gene surveys and/or visualization techniques to determine the nature and holobiont site of bacterial association, in particular since any metaorganism (configuration) is specific to a time and place and not static given the temporal ('fluidic') nature of host-microbial interactions<sup>97</sup>. The statistical power, with only the few representative genomes available from cultures (as in this study), is therefore not going to be the most reliable to generate concrete conclusions, so we should take these results more qualitatively and with caution. This is especially so, as many of the cultivable fraction may not even be the dominant members of the coral microbiome.

We end by highlighting the importance and need for a global initiative, to create an online catalogue of genomic and physiological features of cultured coral-associated bacteria. Combining the use of these genomic insights with innovative culturing techniques<sup>30</sup>, aimed at improving the collection of coral-associated bacterial isolates will see this field of coral biology surge forward. Such an initiative should likely start with those microbes which have their complete genomes sequenced. This study pioneers the organization of this global collection, as part of the efforts from the Beneficial Microbes for Marine Organisms network (BMMO), through a public invitation to researchers working in this field. As a result, we have here provided a list of all the cultured bacteria from all types of corals that are currently available in public databases, plus isolates that were kept in collections from all the labs that have attended our invitation (**Supplementary Table 1** and available now, open access via [isolates.reefgenomics.org](https://isolates.reefgenomics.org)). Now other researchers can access this virtual collection and/or contact specific labs for collaborations or solicitations of specific microbial strains.

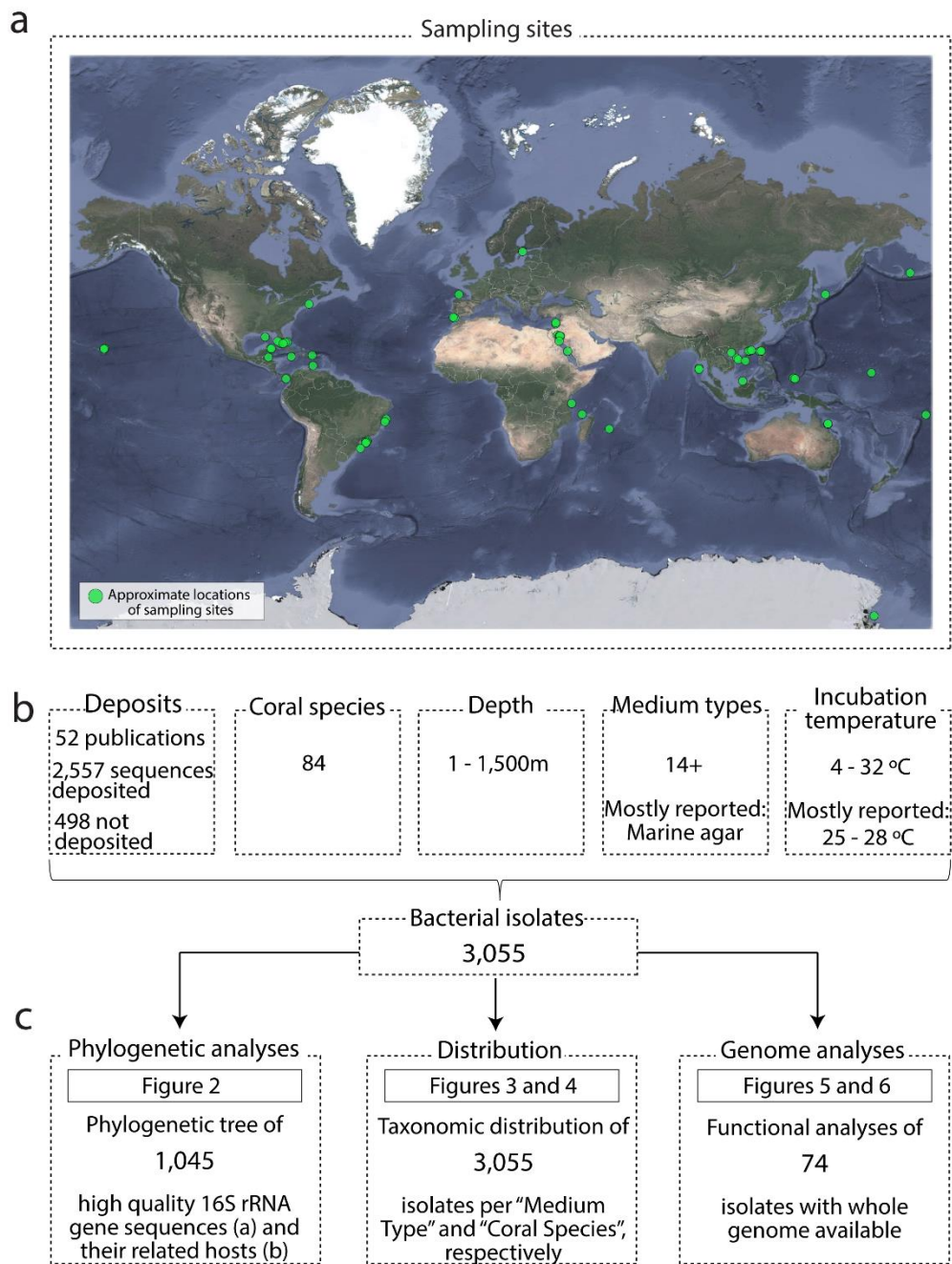
## **Acknowledgments**

Part of this research was carried out in association with the ongoing R&D project registered as ANP 21005-4, "PROBIO-DEEP - Survey of potential impacts caused by oil and gas exploration on deep-sea marine holobionts and selection of potential bioindicators and bioremediation processes for these ecosystems" (UFRJ/Shell Brasil/ANP), sponsored by Shell Brasil under the ANP R&D levy as "Compromisso de Investimentos com Pesquisa e Desenvolvimento". This research project won the Great Barrier Reef Foundation's Out of the

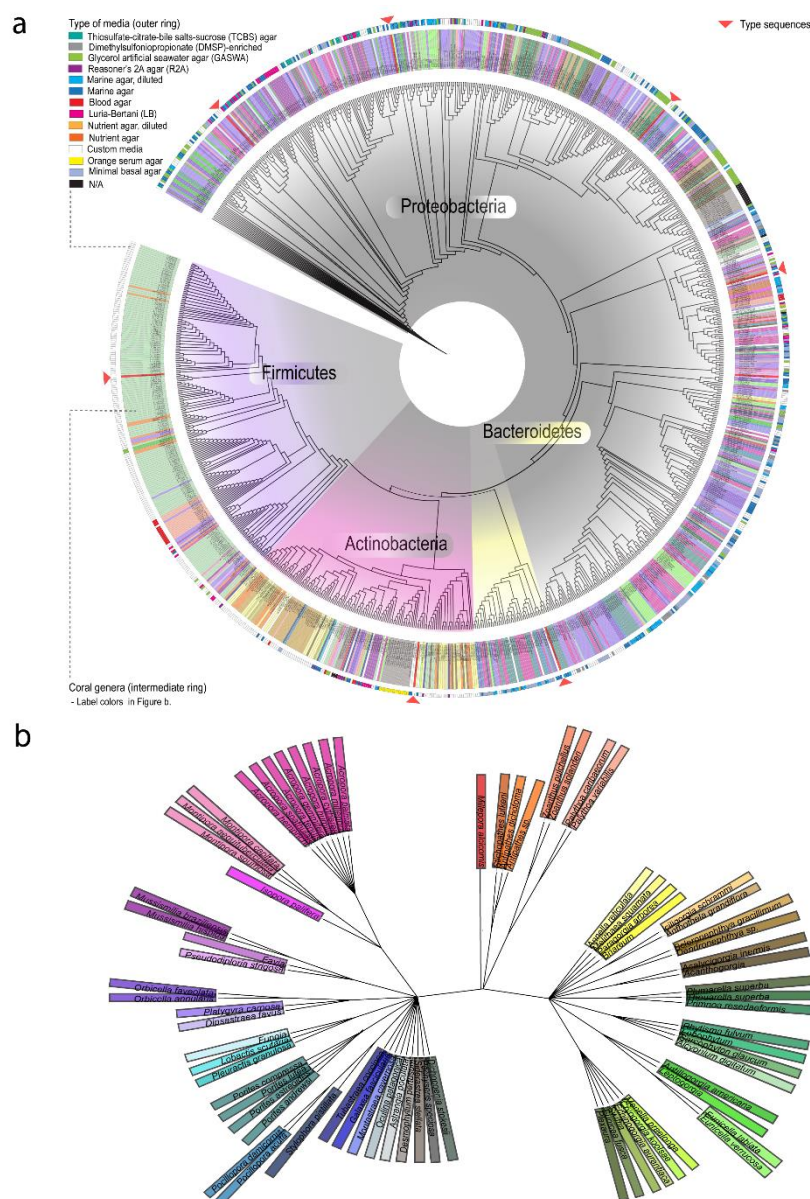
Blue Box Reef Innovation Challenge People's Choice Award supported by The Tiffany & Co. Foundation. The Institute of Bioengineering and Biosciences acknowledges funding provided by the Portuguese Foundation for Science and Technology (FCT) and the European Regional Development Fund (ERDF) through the Grant UIDB/04565/2020. Part of this work was supported by the research grant FA\_05\_2017\_032 conceded to RC and TK-C by the Portuguese Ministry of the Sea (Direção Geral de Política do Mar) under the programme "Fundo Azul". TKC is the recipient of a Research Scientist contract conceded by FCT (CEECIND/00788/2017). N.Z. and K.S. were supported in part by the INBRE-NIGMS of the NIH grant #P20GM103430.

528    **Legends to Figures**

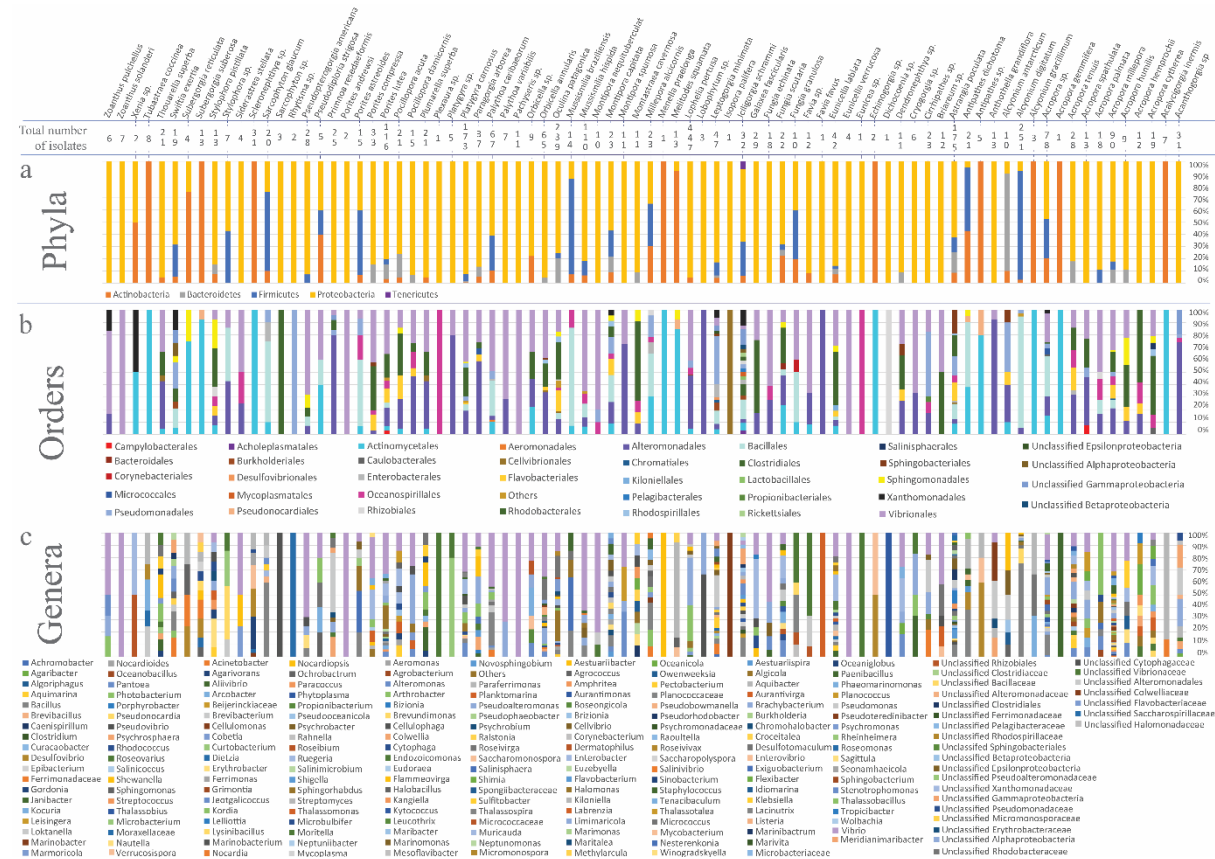
529    **Figure 1. Overview of the data used and generated in this manuscript.** Sampling sites of the  
530 coral species used as isolation sources (a). Data summary recovered from the publications  
531 and accession numbers available in data banks (b). Overview of the analyses performed in the  
532 current manuscript using the available isolates.



**Figure 2. Phylogenetic trees of bacterial strains and coral species. (A)** 16S rRNA gene-based phylogenetic inference of 1,045 coral-associated bacterial isolates, plus eight type-strains representing the species *Vibrio alginolyticus*, *Vibrio bivalvicida*, *Pseudoalteromonas aestuarii*, *Pseudomonas guariconensis*, *Massilia namucunensis*, *Vibrionimonas magnilacii*, *Mycetocola tolaasinivorans*, and *Bacillus subtilis*. The outer ring groups, by colour, the medium used to isolate the strains and the inner ring labels the coral genera used as sources for bacterial isolation. **(B)** Phylogenetic tree of the species of corals used in this study produced via <https://www.ncbi.nlm.nih.gov/Taxonomy/CommonTree/wwwcmt.cgi>. The label colours used to identify the genera are linked to the inner ring of Figure 1A.



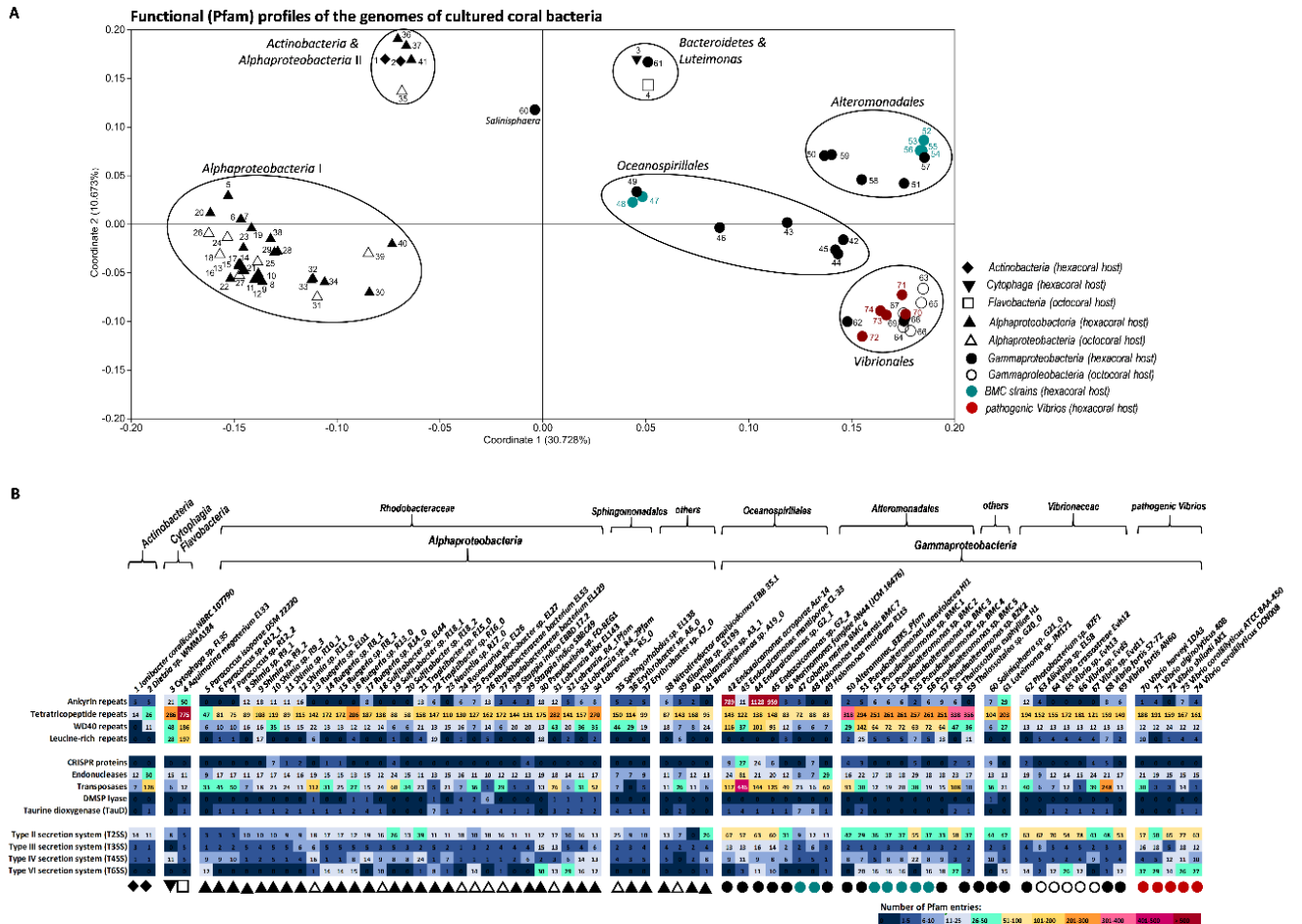
**Figure 3. Phylum (A), order (B) and genus (C) -level profiles of coral-associated bacteria isolated from each coral species.** Taxa (i.e. orders and genera) representing less than 1% of the total percentage of isolates were pulled together and classified as “others”.



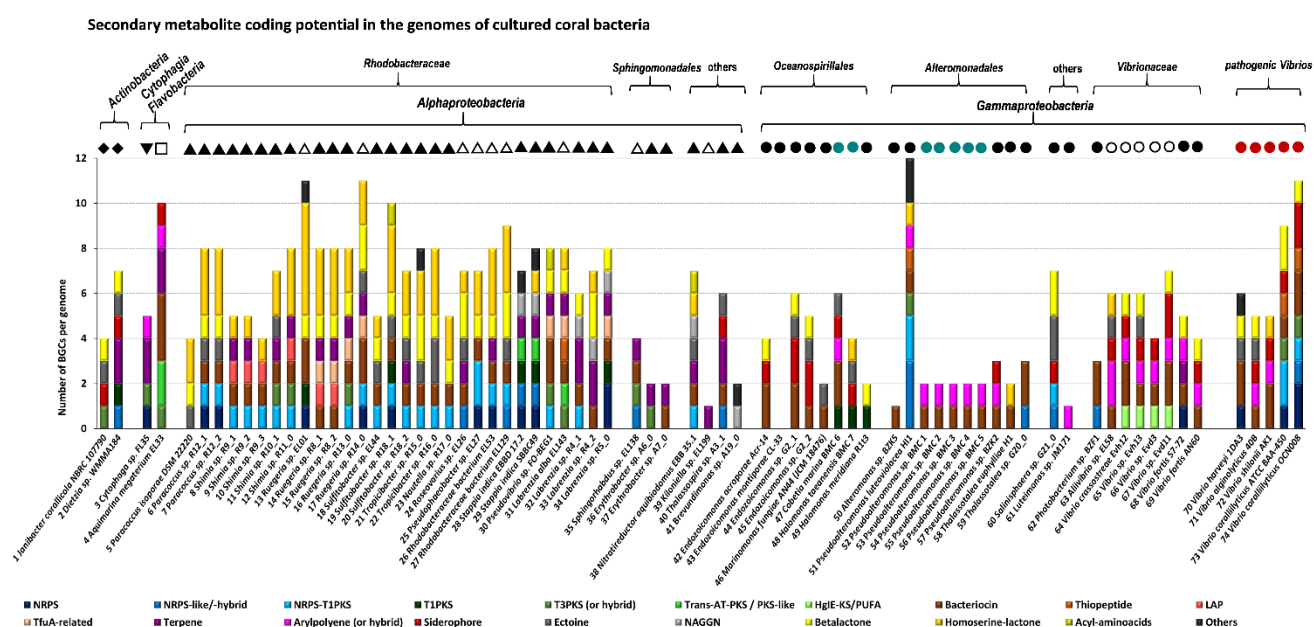
**Figure 4. Phylum (A), order (B,) and genus (C) -level profiles of coral-associated bacteria isolated from each type of culture medium. Taxa (i.e. orders and genera) representing less than 1% of the total percentage of isolates were pulled together and classified as “others”.**



**Figure 5. Functional analysis of 74 genomes of cultured coral bacteria according to their protein family (Pfam) profiles.** Principal coordinates analysis (PCoA) was performed on the Pfam profiles using the Bray-Curtis similarity matrix calculated from Hellinger-transformed abundance data (A). The ordination is shown in Eigenvalue-scale. Symbol shapes indicate the taxonomic class of each genome and the host origin (filled symbols – Scleractinian corals; open symbols - Octocorals). In addition, BMC bacteria are highlighted in cyan blue while typical coral pathogens are highlighted in dark red. Isolate numbers (as in panel B) are given next to each symbol. The number of CDSs assigned to Pfam entries related to Eukaryotic-like proteins “ELPs” (i.e. ankyrin-, tetratricopeptide-, WD40- and leucine-rich repeats) and other features involved in host-microbe interactions are highlighted in the table below (B). The colour code from dark blue to dark red reflects an increase in the number of CDSs related to each function. ELPs, CRISPR proteins, endonucleases, transposases and secretion systems were each represented by more than one Pfam entry across the dataset. The CDS counts of these functionally belonging Pfams were summed. The number of Pfams that contributed to each function were as follows: ankyrin repeats – 5 Pfam entries; tetratricopeptide repeats - 21 Pfam entries; WD40 repeats – 6 Pfam entries; leucine-rich repeats – 8 Pfam entries ; CRISPR proteins – 21 Pfam entries; endonucleases – 42 Pfam entries; transposases – 37 Pfam entries; T2SS – 17 Pfam entries; T3SS – 19 Pfam entries; T4SS – 15 Pfam entries; T6SS – 18 Pfam entries. In the case of taurine and dimethylsulfoniopropionate (DMSP) catabolism only one Pfam entry (PF02668.16 and PF16867.5) was found, respectively.



**Figure 6. Distribution of biosynthetic gene clusters (BGCs) across 74 genomes of cultured coral bacteria.** BGC counts per compound class were obtained using antiSMASH v.5.0 with default settings (and all extra features on). NAGGN - N-acetylglutaminyglutamine amide; LAP - linear azol(in)e-containing peptide; hglE-KS- heterocyst glycolipid synthase-like PKS; PUFA- polyunsaturated fatty acids; NRPS - non-ribosomal peptide synthetase cluster; PKS – polyketide synthase cluster; TfuA-related - TfuA-related ribosomal peptides. The category "others" comprises rare BGCs that had each less than three entries across the dataset (among those were furan, ladderane-hybrid, phosphonate, polybrominated diphenyl ethers, lasso peptide, lanthipeptide and butyrolactone BGCs). Symbol shapes above bars indicate the taxonomic class and the host origin of each genome (as in Figure 5).



## References

1. Bosch, T. C. G. & McFall-Ngai, M. J. Metaorganisms as the new frontier. *Zoology* vol. 114 185–190 (2011).
2. Doolittle, F. & Inkpen, A. S. Processes and patterns of interaction as units of selection: An introduction to ITSNTS thinking. *Proceedings of the National Academy of Sciences of the United States of America* vol. 115 4006–4014 (2018).
3. Moran, N. A. & Sloan, D. B. The Hologenome Concept: Helpful or Hollow? *PLoS Biol.* **13**, e1002311 (2015).
4. van Oppen, M. J. H. & Medina, M. Coral evolutionary responses to microbial symbioses. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **375**, 20190591 (2020).
5. Rohwer, F., Seguritan, V., Azam, F. & Knowlton, N. Diversity and distribution of coral-

615 associated bacteria. *Mar. Ecol. Prog. Ser.* **243**, 1–10 (2002).

616 6. Littman, R. A., Willis, B. L., Pfeffer, C. & Bourne, D. G. Diversities of coral-associated bacteria  
617 differ with location, but not species, for three acroporid corals on the Great Barrier Reef.  
618 *FEMS Microbiol. Ecol.* **68**, 152–163 (2009).

619 7. Hernandez-Agreda, A., Leggat, W. & Ainsworth, T. D. A place for taxonomic profiling in the  
620 study of the coral prokaryotic microbiome. *FEMS Microbiol. Lett.* **366**, (2019).

621 8. Robbins, S. J. *et al.* A genomic view of the reef-building coral *Porites lutea* and its microbial  
622 symbionts. *Nature Microbiology* vol. 4 2090–2100 (2019).

623 9. Huggett, M. J. & Apprill, A. Coral microbiome database: Integration of sequences reveals high  
624 diversity and relatedness of coral-associated microbes. *Environ. Microbiol. Rep.* **11**, 372–385  
625 (2019).

626 10. Ziegler, M., Seneca, F. O., Yum, L. K., Palumbi, S. R. & Voolstra, C. R. Bacterial community  
627 dynamics are linked to patterns of coral heat tolerance. *Nat. Commun.* **8**, 14213 (2017).

628 11. Shibl, A. A. *et al.* Diatom modulation of select bacteria through use of two unique secondary  
629 metabolites. *Proc. Natl. Acad. Sci.* **117**, 202012088 (2020).

630 12. Romano, S., Schulz-Vogt, H. N., González, J. M. & Bondarev, V. Phosphate limitation induces  
631 drastic physiological changes, virulence-related gene expression, and secondary metabolite  
632 production in *Pseudovibrio* sp. strain FO-BEG1. *Appl. Environ. Microbiol.* **81**, 3518–3528  
633 (2015).

634 13. Raina, J. B. *et al.* Isolation of an antimicrobial compound produced by bacteria associated  
635 with reef-building corals. *PeerJ* **2016**, e2275 (2016).

636 14. Neave, M. J., Michell, C. T., Apprill, A. & Voolstra, C. R. *Endozoicomonas* genomes reveal  
637 functional adaptation and plasticity in bacterial strains symbiotically associated with diverse  
638 marine hosts. *Sci. Rep.* **7**, 1–12 (2017).

639 15. Karimi, E. *et al.* Genomic blueprints of sponge-prokaryote symbiosis are shared by low  
640 abundant and cultivatable Alphaproteobacteria. *Sci. Rep.* **9**, 1–15 (2019).

641 16. Staley, J. T. & Konopka, A. Measurement of in Situ Activities of Nonphotosynthetic  
642 Microorganisms in Aquatic and Terrestrial Habitats. *Annu. Rev. Microbiol.* **39**, 321–346  
643 (1985).

644 17. Amann, R. L., Ludwig, W. & Schleifer, K. H. Phylogenetic identification and in situ detection of  
645 individual microbial cells without cultivation. *Microbiological Reviews* vol. 59 143–169 (1995).

646 18. Hugenholtz, P., Goebel, B. M. & Pace, N. R. Impact of culture-independent studies on the  
647 emerging phylogenetic view of bacterial diversity. *Journal of Bacteriology* vol. 180 6793  
648 (1998).

649 19. Stewart, E. J. Growing unculturable bacteria. *J. Bacteriol.* **194**, 4151–4160 (2012).

650 20. Suzuki, M. T. *et al.* Bacterial diversity among small-subunit rRNA gene clones and cellular  
651 isolates from the same seawater sample. *Appl. Environ. Microbiol.* **63**, 983–989 (1997).

652 21. Lagier, J. C. *et al.* Current and past strategies for bacterial culture in clinical microbiology. *Clin.*  
653 *Microbiol. Rev.* **28**, 208–236 (2015).

- 654 22. Pham, V. H. T. & Kim, J. Cultivation of unculturable soil bacteria. *Trends in Biotechnology* vol.  
655 30 475–484 (2012).
- 656 23. Pogoreutz, C. & Voolstra, C. R. Isolation, culturing, and cryopreservation of *Endozoicomonas*  
657 (Gammaproteobacteria: Oceanospirillales: Endozoicomonadaceae) from reef-building corals.  
658 *protocols.io* (2018) doi:10.17504/protocols.io.t2aeqae.
- 659 24. Raina, J. B., Tapiolas, D., Willis, B. L. & Bourne, D. G. Coral-associated bacteria and their role in  
660 the biogeochemical cycling of sulfur. *Appl. Environ. Microbiol.* **75**, 3492–3501 (2009).
- 661 25. Keller-Costa, T. *et al.* The gorgonian coral *Eunicella labiata* hosts a distinct prokaryotic  
662 consortium amenable to cultivation. *FEMS Microbiol. Ecol.* **93**, 143 (2017).
- 663 26. Sweet, M. J., Croquer, A. & Bythell, J. C. Bacterial assemblages differ between compartments  
664 within the coral holobiont. *Coral Reefs* **30**, 39–52 (2011).
- 665 27. Pollock, F. J. *et al.* Coral-associated bacteria demonstrate phylosymbiosis and cophylogeny.  
666 *Nat. Commun.* **9**, 4921 (2018).
- 667 28. Pernice, M. *et al.* Down to the bone: the role of overlooked endolithic microbiomes in reef  
668 coral health. *ISME Journal* vol. 14 325–334 (2020).
- 669 29. Sweet, M. J. & Bulling, M. T. On the Importance of the Microbiome and Pathobiome in Coral  
670 Health and Disease. *Front. Mar. Sci.* **4**, 9 (2017).
- 671 30. Modolon, F., Barno, A. R., Villela, H. D. M. & Peixoto, R. S. Ecological and biotechnological  
672 importance of secondary metabolites produced by coral-associated bacteria. *Journal of*  
673 *Applied Microbiology* jam.14766 (2020) doi:10.1111/jam.14766.
- 674 31. Disalvo, L. H. Isolation of bacteria from the corallum of *Porites lobata* (Vaughn) and its  
675 possible significance. *Integr. Comp. Biol.* **9**, 735–740 (1969).
- 676 32. Ducklow, H. W. & Mitchell, R. Bacterial populations and adaptations in the mucus layers on  
677 living corals. *Limnol. Oceanogr.* **24**, 715–725 (1979).
- 678 33. Aronson, R. B. & Precht, W. F. White band diseases and the changing face of Caribbean coral  
679 reefs. *Hydrobiologia* **460**, 25–38 (2001).
- 680 34. Peters, E. C., Oprandy, J. J. & Yevich, P. P. Possible causal agent of “white band disease” in  
681 caribbean acroporid corals. *J. Invertebr. Pathol.* **41**, 394–396 (1983).
- 682 35. Chet, I. & Mitchell, R. Ecological aspects of microbial chemotactic behavior. *Annual review of*  
683 *microbiology* vol. 30 221–239 (1976).
- 684 36. Antonius, A. Coral Reef Pathology: A Review. *Proceedings of the 4th International Coral Reef*  
685 *Symposium* vol. 2 3–6 (1981).
- 686 37. Kushmaro, A., Rosenberg, E., Fine, M. & Loya, Y. Bleaching of the coral *Oculina patagonica* by  
687 *Vibrio* AK-1. *Mar. Ecol. Prog. Ser.* **147**, 159–165 (1997).
- 688 38. Rozenblat, Y. B.-H. & Rosenberg, E. Temperature-Regulated Bleaching and Tissue Lysis of  
689 *Pocillopora damicornis* by the Novel Pathogen *Vibrio coralliilyticus*. in *Coral Health and*  
690 *Disease* 301–324 (Springer Berlin Heidelberg, 2004). doi:10.1007/978-3-662-06414-6\_17.
- 691 39. Vidal-Dupiol, J. *et al.* Physiological responses of the scleractinian coral *Pocillopora damicornis*  
692 to bacterial stress from *Vibrio coralliilyticus*. *J. Exp. Biol.* **214**, 1533–1545 (2011).

- 693 40. Ushijima, B. *et al.* *Vibrio coralliilyticus* strain OCN008 is an etiological agent of acute  
694 montipora white syndrome. *Appl. Environ. Microbiol.* **80**, 2102–2109 (2014).
- 695 41. Bourne, D. G. *et al.* Microbial disease and the coral holobiont. *Trends in Microbiology* vol. 17  
696 554–562 (2009).
- 697 42. Reshef, L., Koren, O., Loya, Y., Zilber-Rosenberg, I. & Rosenberg, E. The Coral Probiotic  
698 Hypothesis. *Environ. Microbiol.* **8**, 2068–2073 (2006).
- 699 43. Rosenberg, E., Koren, O., Reshef, L., Efrony, R. & Zilber-Rosenberg, I. The role of  
700 microorganisms in coral health, disease and evolution. *Nature Reviews Microbiology* vol. 5  
701 355–362 (2007).
- 702 44. Rosado, P. M. *et al.* Marine probiotics: increasing coral resistance to bleaching through  
703 microbiome manipulation. *ISME J.* **13**, 921–936 (2019).
- 704 45. Voolstra, C. R. & Ziegler, M. Adapting with Microbial Help: Microbiome Flexibility Facilitates  
705 Rapid Responses to Environmental Change. *BioEssays* **42**, 2000004 (2020).
- 706 46. Ritchie, K. B. Regulation of microbial populations by coral surface mucus and mucus-  
707 associated bacteria. *Mar. Ecol. Prog. Ser.* **322**, 1–14 (2006).
- 708 47. Shnit-Orland, M. & Kushmaro, A. Coral mucus-associated bacteria: A possible first line of  
709 defense. *FEMS Microbiol. Ecol.* **67**, 371–380 (2009).
- 710 48. Kuek, F. W. I. *et al.* The potential roles of bacterial communities in coral defence: A case study  
711 at Talang-talang reef. *Ocean Sci. J.* **50**, 269–282 (2015).
- 712 49. Romano, S. Ecology and biotechnological potential of bacteria belonging to the genus  
713 *Pseudovibrio*. *Applied and Environmental Microbiology* vol. 84 (2018).
- 714 50. Hinger, I., Ansorge, R., Musmann, M. & Romano, S. Phylogenomic analyses of members of  
715 the widespread marine heterotrophic genus *pseudovibrio* suggest distinct evolutionary  
716 trajectories and a novel genus, *polycladidibacter* gen. nov. *Appl. Environ. Microbiol.* **86**,  
717 (2020).
- 718 51. Work, T. & Meteyer, C. To Understand Coral Disease, Look at Coral Cells. *Ecohealth* **11**, 610–  
719 618 (2014).
- 720 52. Peixoto, R. S., Sweet, M. & Bourne, D. G. Customized Medicine for Corals. *Front. Mar. Sci.* **6**,  
721 686 (2019).
- 722 53. Peixoto, R., Rosado, P., Leite, D. & Rosado, A. S. Beneficial Microorganisms for Corals (BMC):  
723 proposed mechanisms for coral health and resilience. *Front. Microbiol.* **8**, 341 (2017).
- 724 54. Schloss, P. D. *et al.* Introducing mothur: Open-source, platform-independent, community-  
725 supported software for describing and comparing microbial communities. *Appl. Environ.*  
726 *Microbiol.* **75**, 7537–7541 (2009).
- 727 55. Quast, C. *et al.* The SILVA ribosomal RNA gene database project: Improved data processing  
728 and web-based tools. *Nucleic Acids Res.* **41**, D590–D596 (2013).
- 729 56. Sayers, E. W. *et al.* Database resources of the national center for biotechnology information.  
730 *Nucleic Acids Research* vol. 37 3124 (2009).
- 731 57. Letunic, I. & Bork, P. Interactive Tree of Life (iTOL) v4: Recent updates and new

732 developments. *Nucleic Acids Res.* **47**, W256–W259 (2019).

733 58. Overbeek, R. *et al.* The SEED and the Rapid Annotation of microbial genomes using  
734 Subsystems Technology (RAST). *Nucleic Acids Res.* **42**, D206–14 (2014).

735 59. Wu, S., Zhu, Z., Fu, L., Niu, B. & Li, W. WebMGA: A customizable web server for fast  
736 metagenomic sequence analysis. *BMC Genomics* **12**, 444 (2011).

737 60. Hammer, Ø., Harper, D. A. T. & Ryan, P. D. Past: Paleontological statistics software package  
738 for education and data analysis. *Palaeontol. Electron.* **4**, 178 (2001).

739 61. Blin, K. *et al.* AntiSMASH 5.0: Updates to the secondary metabolite genome mining pipeline.  
740 *Nucleic Acids Res.* **47**, W81–W87 (2019).

741 62. Nguyen, M. T. H. D., Liu, M. & Thomas, T. Ankyrin-repeat proteins from sponge symbionts  
742 modulate amoebal phagocytosis. *Mol. Ecol.* **23**, 1635–1645 (2014).

743 63. Karimi, E. *et al.* Comparative metagenomics reveals the distinctive adaptive features of the  
744 *Spongia officinalis* endosymbiotic consortium. *Front. Microbiol.* **8**, (2017).

745 64. Jahn, M. T. *et al.* A Phage Protein Aids Bacterial Symbionts in Eukaryote Immune Evasion. *Cell*  
746 *Host Microbe* **26**, 542–550.e5 (2019).

747 65. Karimi, E. *et al.* Metagenomic binning reveals versatile nutrient cycling and distinct adaptive  
748 features in alphaproteobacterial symbionts of marine sponges. *FEMS Microbiol. Ecol.* **94**,  
749 (2018).

750 66. Engelberts, J. P. *et al.* Characterization of a sponge microbiome using an integrative genome-  
751 centric approach. *ISME J.* **14**, 1100–1110 (2020).

752 67. Botté, E. S. *et al.* Changes in the metabolic potential of the sponge microbiome under ocean  
753 acidification. *Nat. Commun.* **10**, 1–10 (2019).

754 68. Stringlis, I. A., Zamioudis, C., Berendsen, R. L., Bakker, P. A. H. M. & Pieterse, C. M. J. Type III  
755 secretion system of beneficial rhizobacteria *Pseudomonas simiae* WCS417 and *Pseudomonas*  
756 *defensor* WCS374. *Front. Microbiol.* **10**, 1631 (2019).

757 69. Juhas, M., Crook, D. W. & Hood, D. W. Type IV secretion systems: Tools of bacterial horizontal  
758 gene transfer and virulence. *Cellular Microbiology* vol. 10 2377–2386 (2008).

759 70. Lin, L., Lezan, E., Schmidt, A. & Basler, M. Abundance of bacterial Type VI secretion system  
760 components measured by targeted proteomics. *Nat. Commun.* **10**, 1–11 (2019).

761 71. Cianciotto, N. P. & White, R. C. Expanding role of type II secretion in bacterial pathogenesis  
762 and beyond. *Infect. Immun.* **85**, (2017).

763 72. Schöner, T. A. *et al.* Aryl Polyenes, a Highly Abundant Class of Bacterial Natural Products, Are  
764 Functionally Related to Antioxidative Carotenoids. *ChemBioChem* **17**, 247–253 (2016).

765 73. Persson, O. P. *et al.* High abundance of virulence gene homologues in marine bacteria.  
766 *Environ. Microbiol.* **11**, 1348–1357 (2009).

767 74. Romano, S. *et al.* Comparative genomic analysis reveals a diverse repertoire of genes involved  
768 in prokaryote-eukaryote interactions within the *Pseudovibrio* Genus. *Front. Microbiol.* **7**, 387  
769 (2016).

770 75. Cárdenas, A. *et al.* Excess labile carbon promotes the expression of virulence factors in coral  
771 reef bacterioplankton. *ISME J.* **12**, 59–76 (2018).

772 76. Hardoim, C. C. P. & Costa, R. Microbial communities and bioactive compounds in marine  
773 sponges of the family irciniidae-A review. *Marine Drugs* vol. 12 5089–5122 (2014).

774 77. Esteves, A. I. S., Cullen, A. & Thomas, T. Competitive interactions between sponge-associated  
775 bacteria. *FEMS Microbiol. Ecol.* **93**, 8 (2017).

776 78. Blockley, A., Elliott, D. R., Roberts, A. P. & Sweet, M. Symbiotic microbes from marine  
777 invertebrates: Driving a new era of natural product drug discovery. *Diversity* vol. 9 49 (2017).

778 79. Raimundo, I., Silva, S. G., Costa, R. & Keller-Costa, T. Bioactive secondary metabolites from  
779 octocoral-Associated microbes—New chances for blue growth. *Mar. Drugs* **16**, 485 (2018).

780 80. Nguyen-Kim, H. *et al.* High occurrence of viruses in the mucus layer of scleractinian corals.  
781 *Environ. Microbiol. Rep.* **6**, 675–682 (2014).

782 81. Reynolds, D. & Thomas, T. Evolution and function of eukaryotic-like proteins from sponge  
783 symbionts. *Mol. Ecol.* **25**, 5242–5253 (2016).

784 82. Silva, S. G., Blom, J., Keller-Costa, T. & Costa, R. Comparative genomics reveals complex  
785 natural product biosynthesis capacities and carbon metabolism across host-associated and  
786 free-living Aquimarina (Bacteroidetes, Flavobacteriaceae) species. *Environ. Microbiol.* **21**,  
787 4002–4019 (2019).

788 83. Tandon, K., Chiang, P. W., Chen, W. M. & Tang, S. L. Draft genome sequence of  
789 Endozoicomonas acroporae strain Acr-14T, isolated from Acropora coral. *Genome Announc.*  
790 **6**, (2018).

791 84. Roder, C., Bayer, T., Aranda, M., Kruse, M. & Voolstra, C. R. Microbiome structure of the  
792 fungid coral Ctenactis echinata aligns with environmental differences. *Mol. Ecol.* **24**, 3501–  
793 3511 (2015).

794 85. Shnit-Orland, M., Sivan, A. & Kushmaro, A. Antibacterial Activity of Pseudoalteromonas in the  
795 Coral Holobiont. *Microb. Ecol.* **64**, 851–859 (2012).

796 86. Ushijima, B. *et al.* Mutation of the toxR or mshA genes from Vibrio coralliilyticus strain  
797 OCN014 reduces infection of the coral Acropora cytherea. *Environ. Microbiol.* **18**, 4055–4067  
798 (2016).

799 87. Weynberg, K. D., Voolstra, C. R., Neave, M. J., Buerger, P. & Van Oppen, M. J. H. From cholera  
800 to corals: Viruses as drivers of virulence in a major coral bacterial pathogen. *Sci. Rep.* **5**, 1–9  
801 (2015).

802 88. Fang, Z., Sampson, S. L., Warren, R. M., Gey Van Pittius, N. C. & Newton-Foot, M. Iron  
803 acquisition strategies in mycobacteria. *Tuberculosis* vol. 95 123–130 (2015).

804 89. Isaac, D. T., Laguna, R. K., Valtz, N. & Isberg, R. R. MavN is a Legionella pneumophila vacuole-  
805 associated protein required for efficient iron acquisition during intracellular growth. *Proc.*  
806 *Natl. Acad. Sci. U. S. A.* **112**, E5208–E5217 (2015).

807 90. Holmstrom, C. & Kjelleberg, S. Marine Pseudoalteromonas species are associated with higher  
808 organisms and produce biologically active extracellular agents. *FEMS Microbiol. Ecol.* **30**, 285–  
809 293 (2006).

91. Tebben, J. *et al.* Induction of larval metamorphosis of the coral *Acropora millepora* by tetrabromopyrrole isolated from a *Pseudoalteromonas* bacterium. *PLoS One* **6**, e19082 (2011).
92. Sneed, J. M., Sharp, K. H., Ritchie, K. B. & Paul, V. J. The chemical cue tetrabromopyrrole from a biofilm bacterium induces settlement of multiple Caribbean corals. *Proc. R. Soc. B Biol. Sci.* **281**, 20133086 (2014).
93. Eichhorn, E., Van Der Ploeg, J. R., Kertesz, M. A. & Leisinger, T. Characterization of  $\alpha$ -ketoglutarate-dependent taurine dioxygenase from *Escherichia coli*. *J. Biol. Chem.* **272**, 23031–23036 (1997).
94. Desriac, F. *et al.* Bacteriocin as weapons in the marine animal-associated bacteria warfare: Inventory and potential applications as an aquaculture probiotic. *Marine Drugs* vol. 8 1153–1177 (2010).
95. Hols, P., Ledesma-García, L., Gabant, P. & Mignolet, J. Mobilization of Microbiota Commensals and Their Bacteriocins for Therapeutics. *Trends in Microbiology* vol. 27 690–702 (2019).
96. Cimermancic, P. *et al.* Insights into secondary metabolism from a global analysis of prokaryotic biosynthetic gene clusters. *Cell* **158**, 412–421 (2014).
97. Jaspers, C. *et al.* Resolving structure and function of metaorganisms through a holistic framework combining reductionist and integrative approaches. *Zoology* vol. 133 81–87 (2019).

## **Supplementary material**

### **Species of coral used to isolate bacteria**

*Acropora spathulata*, *A. tenuis*, *A. millepora*, *A. cytherea*, *A. humilis*, *A. hemprochii*, *A. gemmifera*, *A. palmata*, *Astrangia poculata*, *Alcyonium digitatum*, *Antipathes sp.*, *Antipathes dichotoma*, *Acalycigorgia inermis*, *Alcyonium antarcticum*, *Anthothella grandiflora*, *Acanthogorgia sp.*,

*Briareum sp.*,

*Cirrhipathe lutkeni*, *Cryogorgia koolsae*,

*Dendronephthya sp.*,

*Eunicella labiata*, *E. verrucosa*,

*Favia fava*, *Fungia granulosa*, *Fungia echinata*, *Fungia scutaria*,

- 842 *Galaxea fascicularis*,
- 843 *Iciligorgia schrammi*, *Isopora palifera*,
- 844 *Montipora spumosa*, *Montipora capitata*, *Montipora aequituberculata*, *Mussismilia hispida*,
- 845 *Mussismilia braziliensis*, *Millepora alcicornis*, *Montastrea cavernosa*, *Menella praelonga*,
- 846 *Melitodes squamata*,
- 847 *Orbicella faveolata*, *Orbicella annularis*, *Oculina patagonica*,
- 848 *Pocilopora damicornis*, *Pocilopora acuta*, *Pachyseris speciose*, *Porites lutea*, *Porites*
- 849 *astreoides*, *Porites compressa*, *Porites andrewsi*, *Pseudodiploria strigosa*, *Palythoa*
- 850 *caribaeorum*, *Palythoa variabilis*, *Platygyra* sp., *Platygyra carnosus*, *Platygyra caribaeorum*,
- 851 *Plexaura* sp., *Pseudopterogorgia americana*, *Paragorgia arborea*, *Plumarella superba*,
- 852 *Primnoa resdaeformis*,
- 853 *Rhytisma fulvum*,
- 854 *Stylophora pistillata*, *Siderastrea stellate*, *Siderastrea siderea*, *Sarcophyton glaucum*,
- 855 *Scleronephthya* sp., *Sarcophyton* sp., *Swiftia exertia*, *Subergorgia suberosa*,
- 856 *Thouarella superba*, *Tubastraea coccinea*,
- 857 *Leptogorgia minimata*, *Lobophytum* sp., *Lophelia pertusa*,
- 858 *Xenia* sp.,
- 859 *Zoanthis solanderi*, *Zoanhus pulchellus*.