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The coral *Oculina patagonica* holobiont and its response to confinement, temperature, and *Vibrio* infections

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Abstract

Background Extensive research on the diversity and functional roles of the microorganisms associated with reef-building corals has been promoted as a consequence of the rapid global decline of coral reefs attributed to climate change. Several studies have highlighted the importance of coral-associated algae (*Symbiodinium*) and bacteria and their potential roles in promoting coral host fitness and survival. However, the complex coral holobiont extends beyond these components to encompass other entities such as protists, fungi, and viruses. While each constituent has been individually investigated in corals, a comprehensive understanding of their collective roles is imperative for a holistic comprehension of coral health and resilience.

Results The metagenomic analysis of the microbiome of the coral *Oculina patagonica* has revealed that fungi of the genera *Aspergillus*, *Fusarium*, and *Rhizofagus* together with the prokaryotic genera *Streptomyces*, *Pseudomonas*, and *Bacillus* were abundant members of the coral holobiont. This study also assessed changes in microeukaryotic, prokaryotic, and viral communities under three stress conditions: aquaria confinement, heat stress, and *Vibrio* infections. In general, stress conditions led to an increase in Rhodobacteraceae, Flavobacteraceae, and Vibrionaceae families, accompanied by a decrease in Streptomycetaceae. Concurrently, there was a significant decline in both the abundance and richness of microeukaryotic species and a reduction in genes associated with antimicrobial compound production by the coral itself, as well as by *Symbiodinium* and fungi.

Conclusion Our findings suggest that the interplay between microeukaryotic and prokaryotic components of the coral holobiont may be disrupted by stress conditions, such as confinement, increase of seawater temperature, or *Vibrio* infection, leading to a dysbiosis in the global microbial community that may increase coral susceptibility to diseases. Further, microeukaryotic community seems to exert influence on the prokaryotic community dynamics, possibly through predation or the production of secondary metabolites with anti-bacterial activity.

Keywords *Oculina patagonica*, Coral, Holobiont, Metagenome, *Vibrio*, Fungi, Heat stress, Aquaria

Introduction

Corals form a dynamic meta-organism known as the coral holobiont, which involves a multipartite relationship between the cnidarian host, its endosymbiotic dinoflagellate algae (family Symbiodiniaceae; [1]), a diverse array of prokaryotes (Archaea and Bacteria), viruses, and eukaryotes (fungi and non-Symbiodiniaceae protists) [2–4]. Symbiodiniaceae, the primary photosymbiont in corals, has long been recognized for their crucial role in

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fulfilling most of the host energy needs, facilitating effective calcification and the formation of modern reefs [5]. Prokaryotes also play a pivotal role in the health and fitness of coral holobionts, and, although most of their specific roles and functions remain unknown, they are involved in nutrient acquisition, production of bioactive secondary metabolites, and probiotic mechanisms such as competitive exclusion of pathogenic bacteria [6].

All these microorganisms coexist and interact with their host, maintaining the coral's health and facilitating its ability to adapt to climate change. When environmental stressors, such as rising temperatures, occur, coral species often lose Symbiodiniaceae algae, leading to coral bleaching due to an energy deficit [7–9]. Additional changes can range from the replacement of mutualistic species by commensalism or parasitic species, as well as an increase in potential pathogens such as *Vibrio* species [10–12]. The simultaneous occurrence of these changes makes it difficult to determine whether the variations in microbial composition in thermally stressed corals are a consequence or cause of bleaching.

The variability observed in coral microbial composition across seasons and spatial scales poses a significant challenge in unraveling the specific roles of microbial species within the coral holobiont under natural conditions. Consequently, many studies aiming to reduce complexity conduct experiments under controlled conditions, such as responses to pH gradients, nutrient fluxes, or thermal stress [13–16]. However, the applicability of data obtained from these controlled settings to natural conditions is still not well-understood. For example, in coral maintained in aquaria, some taxa rapidly decline, or even disappear, while other microorganisms remain unaffected for extended periods [17, 18]. This phenomenon has also been observed in incubations of seawater samples, where a reduction in microbial diversity occurs, accompanied by the replacement of autotrophic microbes with heterotrophs [19–21]. Therefore, within confined corals, additional factors, not well-understood and beyond nutrient imbalances or temperature variations, may contribute to the observed variations.

Omics tools have revolutionized coral research, allowing for a deeper understanding of the various partnerships within the coral holobiont. However, metagenomic approaches aiming to describe the entire holobiont face technical challenges, particularly due to the limited availability of genomes for individual holobiont components, especially microeukaryotes. In fact, only a few coral metagenomes have been sequenced, and most of these studies have analyzed specific partnerships separately, such as focusing on only the prokaryotic [22, 23] or the viral community [24].

In this work, we conducted a metagenomic analysis of the entire *Oculina patagonica* microbiome. This coral, known as an opportunistic colonizer, is currently expanding throughout the Mediterranean Sea coast [25–27]. Its expansion is attributed in part to its broad tolerance for varying light levels and trophic conditions [28, 29], as well as its heightened thermal resilience compared to other scleractinian corals [27, 30]. However, *O. patagonica* is still susceptible to high temperature, which can lead to changes in the coral microbiome, coral bleaching, and, in some cases, irreversible mortality [28, 31–33]. In the Western Mediterranean Sea, these coral bleaching episodes have been linked to the presence of two *Vibrio* species (*Vibrio coralliilyticus* and *Vibrio mediterranei*), whose pathogenicity increases when both bacteria infect the coral simultaneously [28].

The main objective of this study is to provide the first characterization of the *O. patagonica* microbiome and to investigate its changes in response to three different types of stress (confinement, thermal stress, and the presence of two *Vibrio* pathogens). For this purpose, we first described the *O. patagonica* holobiont in its natural environment and then compared it with that of corals maintained under controlled aquarium conditions. Our study sheds light on how alterations in the microeukaryotic community affect the prokaryotic community and whether these microbiome shifts result in a dysbiosis that may increase disease susceptibility in corals.

Material and methods

O. patagonica samples

Twenty-five fragments, each approximately 5 cm in diameter, of apparently healthy specimens of the coral *O. patagonica*, were collected in June 2016 from the Marine Reserve of Tabarca (Mediterranean Sea, 38° 09′ 60.00″ N 0° 27′ 59.99″ E, Spain) and transported to the laboratory. One coral fragment was used to assess the natural holobiont and will be referred to as the Ocu-2016 dataset. The remaining 24 coral fragments were utilized in the aquaria experiment, as described in Rubio-Portillo et al. (2020) [34]. In brief, corals were distributed in two sets of four tanks, with one set incubated at 20 °C and another set at 28 °C. Each set of tanks contained the following treatments: (I) control corals with no *Vibrio* added; (II) corals inoculated with the *Vibrio* co-culture; (III) corals with a *Vibrio* co-culture growing inside a dialysis membrane, and (IV) corals in the presence of monoculture from each of the two *Vibrio* species growing independently inside a dialysis membrane. Dialysis membranes prevented contact between the coral and the *Vibrio* cells but allowed the free diffusion of molecules with a molecular weight lower than 100 kDa. Vibrios used in this experiment were

V. mediterranei (strain Vic-Oc-097) and *V. corallilyticus* (strain Vic-Oc-068).

DNA extraction and metagenomes sequencing

Coral fragments were gently washed three times with 50 ml of sterile-filtered seawater (SFSW) to remove any adventitious microbes. Coral fragments were broken into small pieces and centrifuged for 3 min at 2900 g (Labofuge 400R, Heraeus instruments) in order to remove the mucus from the coral. After centrifugation, the coral pieces were crushed in SFSW using a mortar and pestle, the $CaCO_3$ skeleton was allowed to settle for 15 min, and the supernatant (i.e., the crushed tissue) was collected and used for DNA extraction. DNA from the crushed coral tissue was extracted using the UltraClean Soil DNA Kit (Mo Bio; Carlsbad, CA, USA) following the manufacturer's instructions for maximum yield.

The *O. patagonica* sample (Ocu-2016) was sequenced using paired-end reads on an Illumina MiSeq (Fisabio, Spain) and also a HiSeq4000 platform (Macrogen, South Corea), with a total of 69 Gb of sequence data. Reads from metagenomes obtained from the aquaria experiment were published previously in [34] to assess *Vibrio* abundance in the corals, but the metagenome assemblages reported herein have not been previously published. These metagenomes samples were sequenced using 150 bp single-end reads on an Illumina HiSeq 2000 platform.

Metagenomic analysis

Raw reads were processed by trimming adaptor sequences and low-quality ends (quality score < 30) using Prinseq [35]. Trimmed reads were used to assess the coverage of metagenomic sequencing with Nonpareil [36]. Subsets of ten million reads from each dataset were randomly separated to perform a taxonomic affiliation using Kaiju [37]. "All versus all" comparison was performed using subsets of one million metagenomic reads and BLASTn (cut-off: 95% nucleotide identity and 70% of query cover alignment length). When a match with the same score was found among the different datasets, it was equally counted for all.

Reads were assembled using MEGAHIT [38] for Ocu-2016 metagenome and meta-SPADES [39] for the aquaria experiments. Due to computational reasons, MEGAHIT was chosen to assemble Ocu-2016 libraries and meta-SPADES for the aquaria metagenomes. However, the rest of the bioinformatic pipeline, including the binning of the contigs, was the same for all the assembled metagenomes. Contigs larger than 5 Kb were selected for metagenome binning using two programs, MetaBAT [40] and MaxBin 2.0 [41]. The final optimized bins were obtained with DAS Tool 1.0 [42]. Genome completeness and putative contamination within the MAGs were determined with Anvi'o v.2.1.0 [43]. According to the standards suggested by Konstantinidis et al. [44], the quality of the MAGs was considered acceptable when completeness was higher than 80% and contamination lower than 5%. MAGs were manually checked for consistent coverage and taxonomy across contigs. The post-curated MAGs were subjected to fast pairwise comparison via a Mash algorithm [45] and grouped into primary clusters at 95% average nucleotide identity (ANI). After this de-replication step, taxonomic affiliation of MAGs was obtained using the GTDB-Tk pipeline, which uses a marker gene set to place genomes in the GTDB reference tree [46]. Gene predictions on the individual reads and also from the contigs of the MAGs were carried out using Prodigal [47]. CDS from the contigs bigger than 1 Kb were annotated with EggNOG v.5.0 [48]. Functional annotation of the predicted ORFs from contigs and MAGs was also performed by BLASTp comparisons against the NCBI nr database, Pfam [49], COG [50], and TIGRFAM [51]. To reconstruct the metabolic potential for the MAGs, GhostKOALA was used [52]. The search for genes encoding biosynthesis of secondary metabolites was performed with antiSMASH v.3.0 [53]. For the identification of putative enzymes involved in the breakdown, biosynthesis or modification of carbohydrates, the CDSs from the contigs bigger than 1 Kb were compared against the CAZy database (dbCAN) [54].

Recruitment analyses

To track the relative abundance of each MAG in the different samples, BBMap was used to map metagenomic short reads to MAG contigs [55]. Matches were filtered for single best alignments, using a minimum of 90% query cover alignment length and 95% nucleotide identity of reads mapping against the reference genome (ANIr). In order to remove biases from highly conserved regions and contig edges, the 80% central truncated average of sequencing depth of all bases (TAD80) was used as described previously [56]. MAG abundance in each metagenomic dataset (as percentage of total community) was calculated as the quotient of the MAG's TAD80 value and the genome equivalents (GE) from MicrobeCensus [57].

In the case of the recruitment analysis of the dinoflagellate genomes against each of the metagenomes, a database containing the following 20 dinoflagellate assemblies was constructed: *Breviolum minutum* Mf 1.05b.01 (GCA_000507305.1), *Symbiodinium microadriaticum* (GCA_001939145.1), *Symbiodinium microadriaticum* (GCA_905231925.1), *Symbiodinium microadriaticum* AJIS2-C2 (GCA_018327485.1), *Symbiodinium* sp. clade A Y106 (GCA_003297005.1), *Symbiodinium* sp. clade C Y103 (GCA_003297045.1), *Symbiodinium kawagutii* (GCA_

009767595.1), *Symbiodinium natans* (GCA_905221605.1), *Symbiodinium tridacnidorum* CCMP2592 (GCA_905221615.1), *Symbiodinium* sp. KB8 *(GCA_905221625.1)*, *Symbiodinium linuchaea* CCMP2456 *(GCA_905221635.1)*, *Symbiodinium pilosum* (GCA_905231905.1), *Symbiodinium necroappetens* (GCA_905231915.1), *Amoebophrya* sp. *AT5.2* (GCA_005223375.1), *Amoebophrya* sp. *A120* (GCA_905178155.14), *Amoebophrya* sp. *A25* (GCA_905178165.1), *Polarella glacialis* (GCA_905237085.1), *Polarella glacialis* (GCA_905237095.1), *Cladocopium goreaui* (GCA_947184155.1), and *Amphidinium carterae* (GCA_019702695.1). BlastN comparisons of subsets of 10 million reads were performed and all the reads that hit over 90% identity with a coverage over 70% were considered. When a read matched several dinoflagellate genomes equally well, it was considered a match for all genomes using a modification of the BlastTab.best_hit_sorted of Enveomics Toolbox. To calculate the frequency of the dinoflagellates in the metagenomes, the number of hits was normalized against the dinoflagellate size dataset.

Comparison with other environmental datasets

For the MetaFast comparison [58], a subset of ten million reads randomly extracted from each collection was first obtained. The metagenome collections used came from Ocu-2016, samples from the aquaria experiments, together with other metagenomes obtained from different corals (*Acropora palmata* (SRR8789034), *Cyphastrea* (Australia, PRJNA364879), *Diploira* (Mexico, PRJNA364874), *Siderastrea* (Mexico, PRJNA364873), a sponge (*Ircinia ramosa*, PRJNA555144), a metagenome from soil (Soil Forest Indian, JGI-Ga0247671), several seawater metagenomes (two from the TARA collections: TARA-009, 5 m, ERR594288 and TARA-009, 55 m, ERR594315) [59]; 4 metagenomes from the MED collection [60]: Med-OCT2015-15 m (SRR5007106); Med-OCT2015-45 m (SRR5007115) and two seawater metagenomes affected by a "bottle-effect," Med-OCT2015-15 m-7 h (SRR8503606) and Med-OCT2015-15 m-14 h (SRR8503605).

Viral sequence analyses

Viral contigs were included if their length was over ≥ 10 kb, and they were identified as a viral sequence of category 1 or 2 by VIRSorter v1.03 [61]. Also, contigs which were identified as prophages by VIRSorter v1.03 were also annotated with PHASTER [62], and only those contigs identified as intact or questionable prophages were used. To cluster viral sequences into viral operational taxonomic units (vOTUs), CD-HIT was used with a cut-off of 95% average nucleotide identity and 80% alignment fraction threshold for the smallest contig [63]. vConTACT2 0.9.09 was used to compare the viral contigs using the Prokaryotic Viral RefSeq version 201 (with ICTV and NCBI taxonomies). Only those connections with a score > 1 were considered. The nets were visualized with Cytoscape [64].

Statistical analysis

Given the absence of replicates to determine the statistical significance of the observed differences between samples, we employed a resampling approach [65]. For this purpose, each pair of samples was combined into a single sample, and this combined sample was then repeatedly split into two random samples of the same size as the originals, a process that was performed 1000 times. The Bray–Curtis (B-C) distance of the two original samples was then compared with the vector of 1000 distances obtained with the resampling. The fraction of distances greater than or equal to the original was taken as an estimate of the p-value for the hypothesis test with the null hypothesis "both samples come from the same community," and the alternative "the two samples do not come from the same community." This fraction thus estimates the probability that, if the two samples came from the same community, the B-C distance would be greater than or equal to that obtained with the two original samples. In all cases, this fraction was 0%, which gives an estimation of p-value < 0.001 that would remain significative after adjusting the whole set of p-values in order to account for multiple testing.

For each pair of samples of interest and for each genus, a proportion comparison test was performed. Depending on the conditions, two types of tests were employed: the parametric chi-square test when the expected frequencies were greater than or equal to 5, and a Monte Carlo chi-square test when any expected frequency was less than 5. Subsequently, p-values for each pair of samples were adjusted using the Benjamini-Yekutieli method, which is ideal for controlling the false discovery rate when the variables are not independent. A genus was considered to have significantly different proportions between samples when adjusted p-value < 0.05.

To evaluate whether the genes encoding the biosynthesis of secondary metabolites were predominantly produced by eukaryotes or prokaryotes, both in Ocu-2016 and in the aquarias, the proportions of genes were compared using chi-square tests and again adjusting the p-values using the Benjamini-Yekutieli method.

All the scripts used for the statistical analysis are available in Supplementary File 1.

Results and discussion

To characterize the coral holobiont community, encompassing eukaryotes, prokaryotes, and viruses, a metagenome of approximately 70 Gb of sequence from a sample of *O. patagonica* collected during the summer of 2016 in Tabarca (Alicante, Spain) was sequenced. This is referred to as the Ocu-2016 dataset. Additionally, to investigate alterations in these communities in response to heat stress or the presence of *Vibrio* coral pathogens, we conducted complementary analyses using published metagenomes from Rubio-Portillo et al. [34]. The published metagenomes came from the same coral sample that had been split into several experimental regimes, though the published analysis primarily focused on characterizing the *Vibrio* assemblages and did not encompass the complete holobiont. For all nine metagenomes, Nonpareil analysis indicated a coverage of approximately 94% for Ocu-2016 and between 50 and 68% for the metagenomes from the aquaria (Supplementary Table 1), indicating a relatively good representation of the biodiversity contained within them.

Oculina patagonica holobiont description

First, to obtain a general overview, a comparison of all *O. patagonica* metagenomes (including the natural and the stressed samples) was conducted against other published coral and Mediterranean seawater datasets. As expected, samples from seawater clustered separately from the coral samples, which formed a cluster comprising metagenomes from *O. patagonica* and other corals (Supplementary Fig. 1).

The reads annotated within the Ocu-2016 coral metagenome were distributed as follows: 10.86% belonged to Bacteria, 3.85% to Eukarya, 0.43% to Archaea, and 0.07% to Viruses, with 84.78% remaining unclassified (Supplementary Table 2). To assess whether this pattern was specific to our sample, a similar analysis was conducted using a dataset from the coral *Porites lutea* (SRR9182857) [23]. The results were comparable, with only 1.4% of the reads identified as eukaryotes and 7.7% classified as prokaryotes (Supplementary Table 2). The low percentage of eukaryotes found suggests that, due to the relatively lower availability of genomes from eukaryotes compared to prokaryotes, some of the unclassified reads may belong to the coral and other microeukaryotic organisms.

O. patagonica associated eukaryotes

Among coral symbionts, Symbiodoniaceae were the first and most important to be recognized, and the mutual transport of nutrients between both taxa has been well described [1]. *O. patagonica* harbors *Symbiodinium* species belonging to three different clades (A, B, and C) from which representative genomes are available (Supplementary Fig. 2). Among them, the genome of *Breviolum minutum* Mf1.05b (also known as *Symbiodinium minutum*), which belongs to clade B, exhibited the highest read recruitment rates. This result was consistent with previous findings that identified *Symbiodinium* type B1 as the primary clade in *O. patagonica* [25]. It is also consistent with prior investigations in other coral species, demonstrating the concurrent association of corals with multiple Symbiodiniaceae, with a prevailing clone [66].

Besides *Symbiodinium*, other protists were also abundant in the *O. patagonica* holobiont, with the Evosea, Euglenozoa, and Apicomplexa phyla being particularly prevalent (Fig. 1A). Previous studies have already documented the presence of other protists in the coral holobiont, suggesting that they may play a role in assisting coral hosts in obtaining sufficient nutrients or serve as an additional food source during recovery from stress, such as tissue loss and bleaching events [67]. Among the detected protists were two Choanoflagellate species, *Monosiga brevicollis* and *Salpingoeca rosetta*, which were not previously known to be associated with corals. The unexpected occurrence of reads matching the parasitic protist *Plasmodium* may have been attributed to the presence of the "ap1icoplast," an organelle in *Plasmodium* and other apicomplexans [68] that is derived from endosymbiotic cyanobacteria. In other coral reef samples, sequences from apicomplexan-related plastids from *Chromera* and *Vitrella* have been consistently detected [69, 70], but given the low recruitment of these genera in Ocu-2016, it is possible that many of the reads matching *Plasmodium* belong to a yet unknown microalgae plastid(s) present in *O. patagonica*.

Numerous non-photosynthetic microeukaryotes associated with corals have been identified, exhibiting diverse roles within the holobiont, ranging from beneficial symbiosis to parasitic relationships, and even acting as primary pathogens [71–73]. In this study, most of the non-photosynthetic microeukaryotic reads were classified as Fungi (Fig. 1A). Among them, the most prominently represented phyla were Ascomycota, Basidiomycota, and Mucoromycota. Aspergillaceae and Glomeraceae were the two major families (Fig. 1B), in good agreement with previous results reporting Ascomycetes and Basidiomycetes as the two major groups of fungi associated with corals [72, 74]. Within the Ascomycota, the genera *Aspergillus* and *Fusarium* were the most abundant (Fig. 1C). A significant abundance of reads assigned to the genus *Rhizophagus* (Glomeraceae family) was also detected. This is a beneficial mycorrhizal fungus commonly used as a soil inoculant in agriculture and forest ecosystems to enhance phosphorus uptake [75]. Recruitment analysis of Ocu-2016 reads

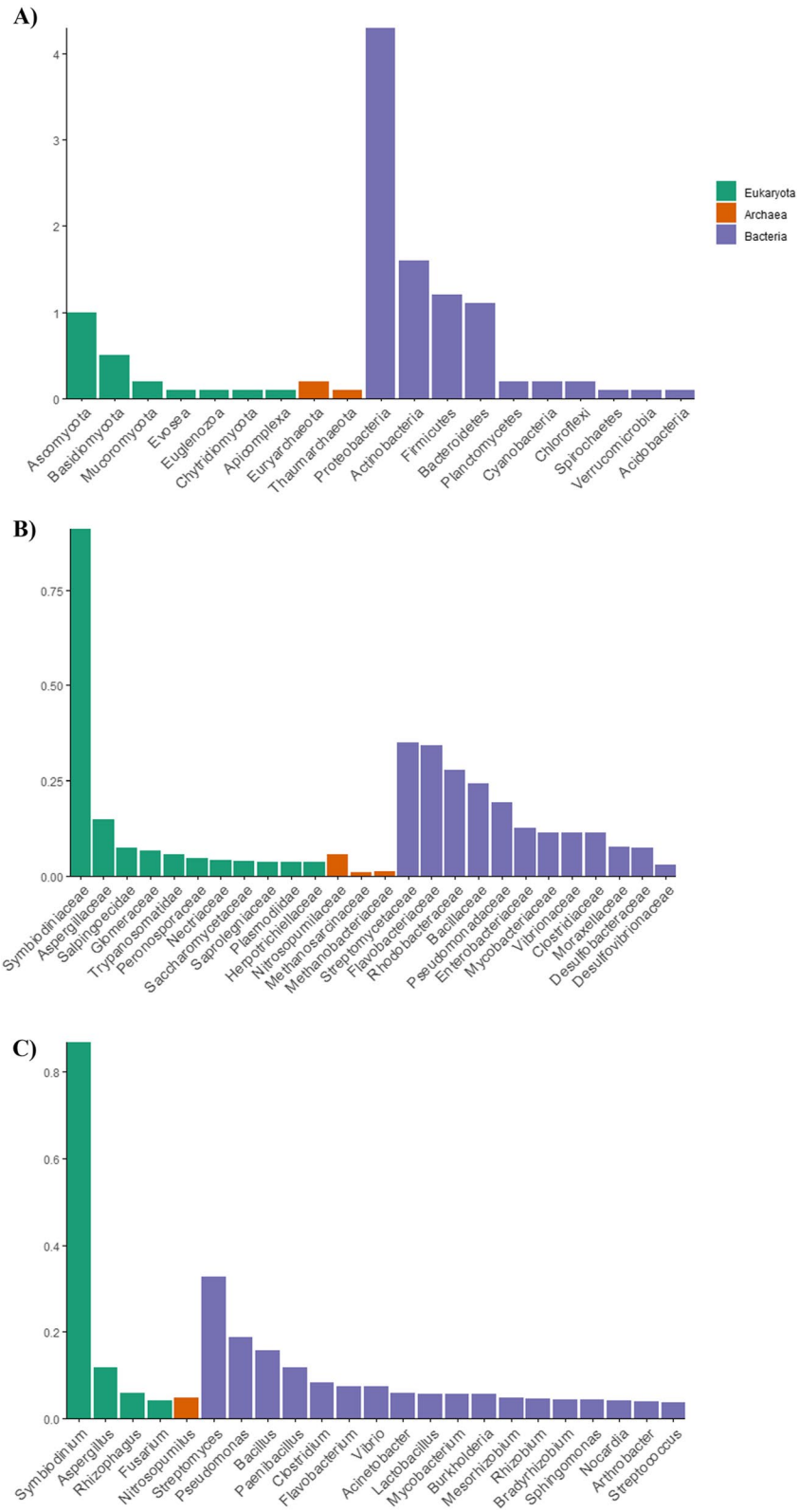

Fig. 1 Microbial taxonomic classification of *O. patagonica* microbiome inferred from metagenomic reads using Kaiju. Taxonomic classification at the phylum (**A**), family (**B**), and genera (**C**) levels

against the reference genome *of Rhizophagus irregularis* (DAOM 181602) revealed the presence of reads with similarity to their ribosomal operons and housekeeping genes. Similar results were also obtained using the microbial metagenomes of the corals *P. lutea* and *Acropora palmata* (results not shown). These findings suggest that although *Rhizophagus* species have not been previously described in aquatic environments, related fungi may contribute beneficial traits to corals.

O. patagonica associated prokaryotes

Among the prokaryotic community, bacterial reads greatly outnumbered those assigned to archaea, maintaining a ratio of approximately 15:1 (Supplementary Table 2). The archaeal community (Fig. 1A) was composed of Euryarchaeota (0.2% of the total reads) and Thaumarchaeota (0.1%). The genus *Nitrosopumilus* exhibited the highest abundance within this family (Fig. 1C). This genus has been previously linked to the crucial ammonium oxidation process occurring within the coral mucus layer [76]. Notably, *Nitrosopumilus* has been previously detected in *O. patagonica*, and its presence has been exclusively associated with healthy colonies [77]. Also in accordance with previous findings obtained by 16S rRNA gene analyses [77], the bacterial reads were primarily composed of Proteobacteria (4.3%), Actinobacteria (1.6%), Firmicutes (1.2%), and Bacteroidetes (1.1%), with *Streptomyces*, *Pseudomonas*, and *Bacillus* as the most abundant genera (Fig. 1).

Binning of the assembled contigs from the Ocu-2016 metagenome resulted in five metagenome-assembled genomes (MAGs), which belonged to Desulfobacterales (MAG1-Ocu2016), Flavobacteriales (MAG2-Ocu2016), Holosporales (MAG3-Ocu2016), Parvularculales (MAG4-Ocu2016), and Rhizobiales (MAG5-Ocu2016) (Supplementary Table 3). These MAGs recruited less than 0.001% of the metagenomics reads, with ANIr values over 99% (Supplementary Table 4), suggesting that only MAGs from low-abundance bacteria displaying a very low intra-population diversity could be retrieved. Excluding MAG1, the average genome size for the other four MAGs from the Ocu-2016 dataset was 1.7 ± 0.5 Mb, a size small enough to be consistent with a host-associated lifestyle, reflecting the loss of non-essential genes [78]. For example, MAG3-Ocu2016 (*Candidatus* Hepatobacter penai; 87.3% complete) lacks key biosynthetic pathways for essential amino acids and shows incomplete synthesis of purines and pyrimidines (Supplementary Table 5). However, it harbors genes for biotin (vitamin B7) synthesis. This could be crucial for the coral holobiont, as both corals and *Symbiodinium* are suggested to be auxotrophs for different B vitamins, potentially obtaining them from associated bacteria [79, 80].

Furthermore, within the MAGs, genes encoding mechanisms for stable symbiosis with the host, such as ankyrin repeats proteins (ARPs), were identified (Supplementary Table 3). ARPs are common protein interaction motifs that modulate intracellular processes, promoting stable symbiotic or pathogenic associations [81]. Previous analysis of ARP distribution in microbial genomes showed that species dedicating more than 0.2% of their protein-coding genes to ARPs are typically obligate intracellular or facultative host-associated species [82]. In this case, MAG3-Ocu2016 and MAG4-Ocu2016, both from the Alphaproteobacteria class, surpass this percentage. Moreover, in the case of MAG4-Ocu2016 (unknown Parvularculales), these proteins were affiliated with other corals and sponges, suggesting its potential as a symbiont for marine invertebrates.

MAG1-Ocu2016 represents a putatively diazotrophic dissimilatory sulfate-reducing bacterium (SRB) belonging to the *Desulfobacter* genus (Supplementary Table 5). The identification of a N_2-fixing SRB, not previously described in corals, carries substantial implications for coral communities that commonly inhabit nutrient-depleted environments. These bacteria may enhance the coral's ability to efficiently convert gaseous nitrogen into a usable form such as ammonia, a vital process for sustaining a steady nitrogen supply for *Symbiodinium*-based primary production within corals [83].

O. patagonica associated virus

A total of 275 different viral operational taxonomic units (vOTUs) were retrieved from Ocu-2016 metagenome, which accounted for 0.08% of the metagenomics reads. Only 14% of these vOTUs exhibited similarity to known reference genomes, and most of them (86%) were classified as dsDNA phages belonging to the class Caudoviricetes (Supplementary Table 6). A phage phylogenetic network using phage reference genomes (Fig. 2) showed that most of the phages identified in the *O. patagonica* holobiont were new. Others grouped mainly with phages infecting *Pseudoalteromonas*, *Pseudomonas*, and *Roseobacter*, three bacterial genera consistently associated with corals. Three vOTUs presented a partial similarity to Suoliviridae sequences (Crassvirales), with 30 to 63% similarity to a major head protein, DNA polymerase or hypothetical proteins. Although marine crassviruses have been previously described [84], *O. patagonica* assembled crassviruses-like clustered separately from them and may thus represent novel lineages.

Five of the identified vOTUs were assigned as eukaryotic viruses belonging to the Phycodnaviridae family within the Nucleocytoplasmic Large DNA Viruses

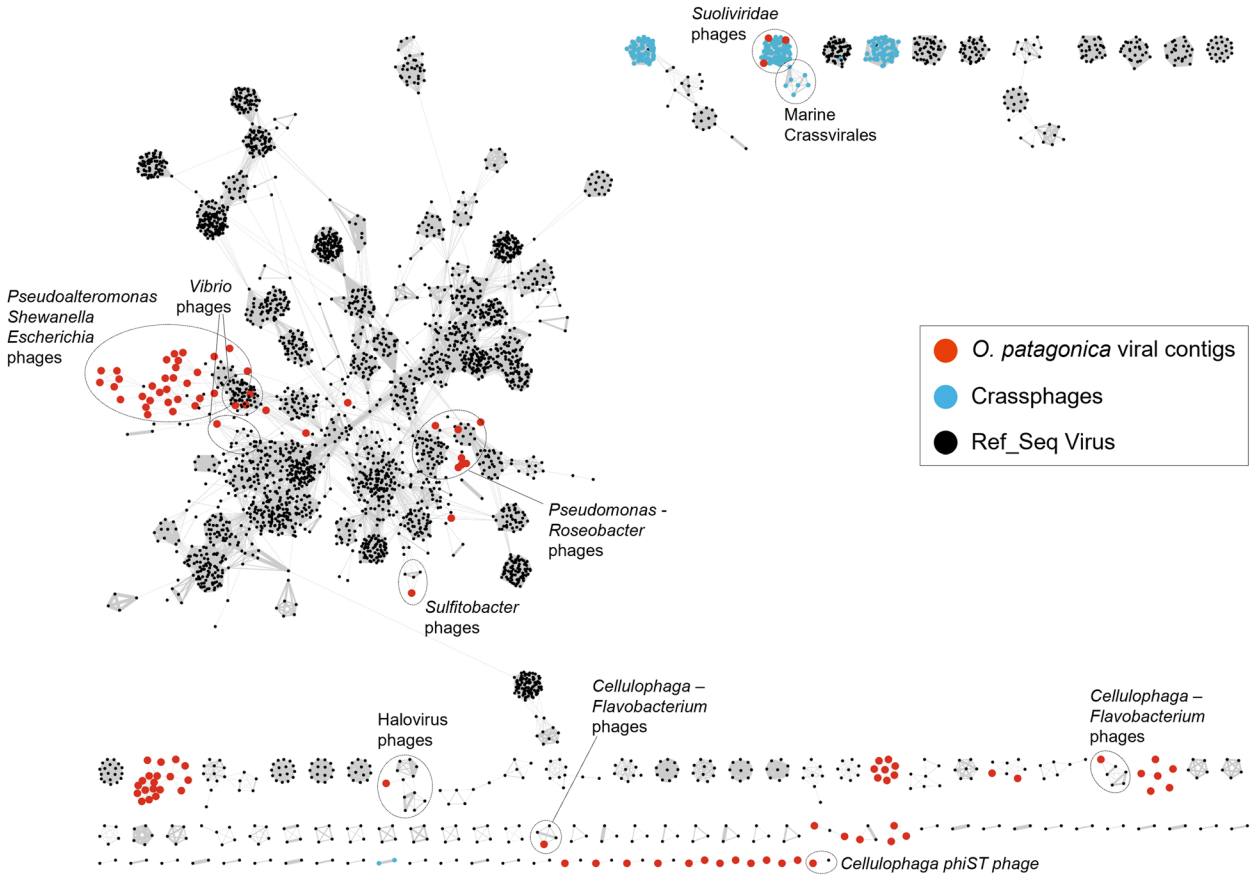

Fig. 2 vConTACT clustering of *O. patagonica* phages and prophages and related prokaryotic Viral RefSeq genomes (version 201). Color nodes represent the *Oculina* predicted viral sequences and black ones the RefSeq virus. Edges represent the vConTACT-generated similarity score between each pair of viruses (only similarity scores of ≥ 1 are included in the network). Highly similar viruses are positioned close together. Only reference viruses that are connected to ≥ 1 predicted phage are included in the network

(NCLDVs) (Supplementary Table 6). The family Phycodnaviridae infects phytoplankton and has been previously detected in both heat-stressed corals and cultures of *Symbiodinium* spp., suggesting that these viruses may have a role in the destruction of algal symbionts or the dysfunction of symbiont–host mutualism, although the extent of such infections is unknown [85–87].

O. patagonica holobiont changes under stress conditions

When the Ocu-2016 dataset (the natural holobiont metagenome) was compared with a collection of the metagenomes derived from the stress experiments, it clustered with the coral samples maintained at 20 °C, and apart from the 28 °C samples (Supplementary Fig. 1). Read-level analysis further supported these findings, with Ocu-2016 sharing 46.9% of reads with C20, only 15% with C28, and lower similarities were observed when compared to the *Vibrio* infection datasets (Fig. 3). Genus-level analysis using a resampling approach showed that coral microbiome composition significantly changed when corals were exposed to the different stress tested in the experiment (Supplementary Table 7). Further, Shannon diversity index was significantly higher in corals maintained in the aquaria compared to the Ocu-2016, mainly in corals under heat stresses and in corals in the presence of vibrios compared to the control ones (pairwise Hutcheson *t*-tests, all adjusted *p*-values 0, Supplementary Table 1). All these results highlight significant shifts in the microbial community of *O. patagonica* under heat stress, particularly in the presence of *Vibrio* species. These community shifts in the aquaria confinements were mainly enrichments of heterotrophic microbes. This phenomenon, known as the "bottle effect," has been previously observed when seawater samples or corals are kept under prolonged confinement conditions [18, 88].

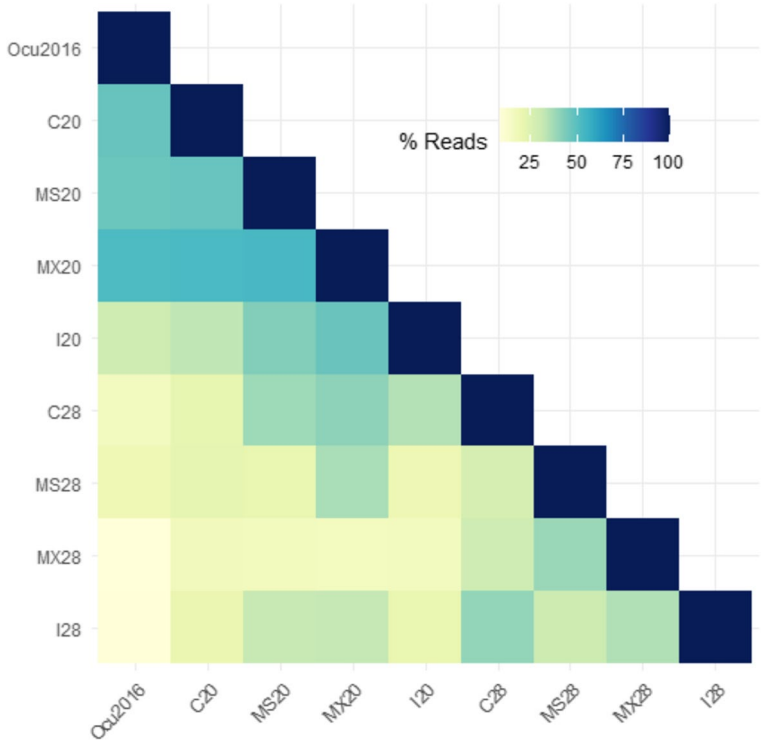

Fig. 3 Sequences shared between metagenomes determined using an "all versus all" comparison of metagenomic reads. Numbers indicate the percentage of shared sequences among datasets

O. patagonica holobiont changes due to aquarium confinement

One of the most extensive effects of confinement was the decrease in the proportion of reads assigned to Eukarya within the metagenome from the coral maintained at 20 °C (from 3.85 to 2.2% compared to Ocu-2016), and the increase of bacterial reads (from 10.85 to 17%). A differential abundance test was used to identify genera with different proportions in Ocu-2016 compared to the coral maintained at 20 °C (Supplementary Table 8). This analysis showed that in the aquaria a decline in *Symbiodinium*, as well as other microeukaryotes and prokaryotes compared to Ocu-2016. Meanwhile, an expansion of the ("natural") rare biosphere was observed under confinement conditions (Fig. 4). Microeukaryotes that proliferated in the aquarium experiments were mainly members of the *Saccharomycodes* genus, which were not detected in Ocu-2016 (Fig. 4). Most of these reads showed similarity to the yeast *Saccharomycodes ludwigii*, with some matching *Hanseniaspora*. The presence of these sugar-consuming microorganisms may be attributed to the increased carbohydrate content in the mucus of stressed corals [89].

Also under confinement conditions, there was a notable increase in certain prokaryotic genera, including potential human pathogens like *Acinetobacter*, *Bordetella*, *Neisseria*, *Klebsiella*, and *Salmonella*, which were not detected in Ocu-2016 (Fig. 4). This shift could be linked to changes in the microeukaryotic community associated with the coral. It has been suggested that microeukaryotes play a role in regulating microbial communities within corals through processes like phagocytosis and the production of antimicrobial compounds [2, 70, 72]. Thus, a search for genes encoding the synthesis of secondary metabolites was conducted to investigate the potential antimicrobial activities of the *O. patagonica* microbiome. Genes encoding polyketide synthases (PKSs) and multienzymatic nonribosomal peptide synthetases (NRPSs) were detected. To determine whether they were predominantly produced by eukaryotes or prokaryotes, we compared the proportions of these genes in both the natural sample and the aquarium samples using the chi-square test. In the natural sample (Ocu-2016), these genes were found almost exclusively in eukaryotes (e.g., *Symbiodinium*, *Scleractinia*, *Blastocladiomycota*, and *Dictyosteliales*), with no bacterial PKSs detected (Fig. 5). Conversely, in the aquarium-maintained corals, these genes were primarily produced by prokaryotic microorganisms. Statistically significant differences were observed between the proportions of eukaryotic and prokaryotic genes (adjusted-p-value < 0.00005), except for the samples C20 and MS20, where the differences

Fig. 4 Prokaryotic genera that showed significant different proportions among treatments. C, control corals; I, infected corals; MS, corals in the presence of *Vibrio* monocultures; MX, corals in the presence of *Vibrio* co-culture. The numerical annotations 20 and 28 refer to corals maintained at 20 °C and 28 °C, respectively

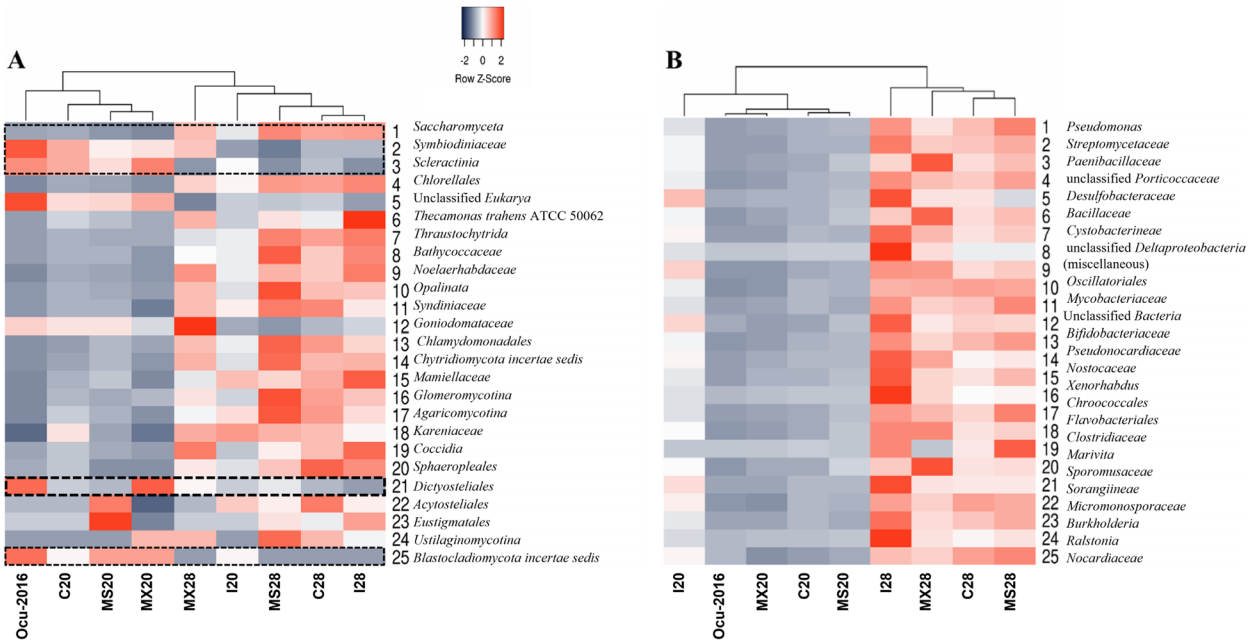

Fig. 5 The taxonomic origins of the predicted polyketide synthase (PKS) clusters in *O. patagonica*. **A** Eukaryotic clusters and **B** bacterial clusters detected. C, control corals; I, infected corals; MS, corals in the presence of *Vibrio* monocultures; MX, corals in the presence of *Vibrio* co-culture. The numerical annotations 20 and 28 refer to corals maintained at 20 °C and 28 °C, respectively

were not statistically significant (adjusted-*p*-value > 0.05). The absence or reduction of these natural eukaryotic PKS could potentially lead to an increase in the proliferation of fast-growing organisms, as discussed below. This would agree with previous results indicating that the coral-associated fungi that decreased under confinement

conditions, such as species from *Aspergillus* or *Fusarium*, displayed antimicrobial activities against human pathogenic bacteria [90, 91].

O. patagonica holobiont changes due to thermal stress under experimental conditions

The proportion of annotated eukaryal reads in the metagenome from the coral maintained at 28 °C remained similar to that at 20 °C. However, there was a substantial increase in the bacterial reads, from 17 to 40%. The number of detected bacterial families also increased, from 112 at 20 °C to 171 at 28 °C (Supplementary Table 2). The differential abundance approach was used to identify genera with different proportions in corals maintained at 20 °C and 28 °C (Supplementary Table 8). This analysis identified various genera from the Rhodobacteraceae family, including *Marivitia*, *Ruegeria*, *Loktanella*, and *Yoonia*, as bacterial bloomers under heat-stress conditions (Fig. 4). In line with our results, the Rhodobacteraceae family has been recently suggested as an indicator species for thermal stress in corals [92]. Furthermore, a significant number of Rhodobacteraceae genera detected in our study are known to be involved in the breakdown of organic sulfur compounds like dimethylsulfoniopropionate (DMSP) and dimethylsulfide (DMS). The production of these compounds increases in corals under thermal stress [93–95]. In good agreement, genes responsible for DMSP catabolism (*dmdA*, *dmdB*, *dmdC*, *dddD*, *dddP*, and *dddL*) increased in abundance in corals kept in aquaria, particularly in corals maintained at 28 °C (from 0.00001% in Ocu-2016 to 0.0001–0.001% of reads). Furthermore, two MAGs recovered from the aquaria metagenomes (MAG9-MS28 and MAG10-MX28) corresponded to two *Rhodobacteraceae* genera potentially involved in DMSP metabolism, *Pelagibaca* and *Yoonia*, respectively (Supplementary Table 3). In fact,

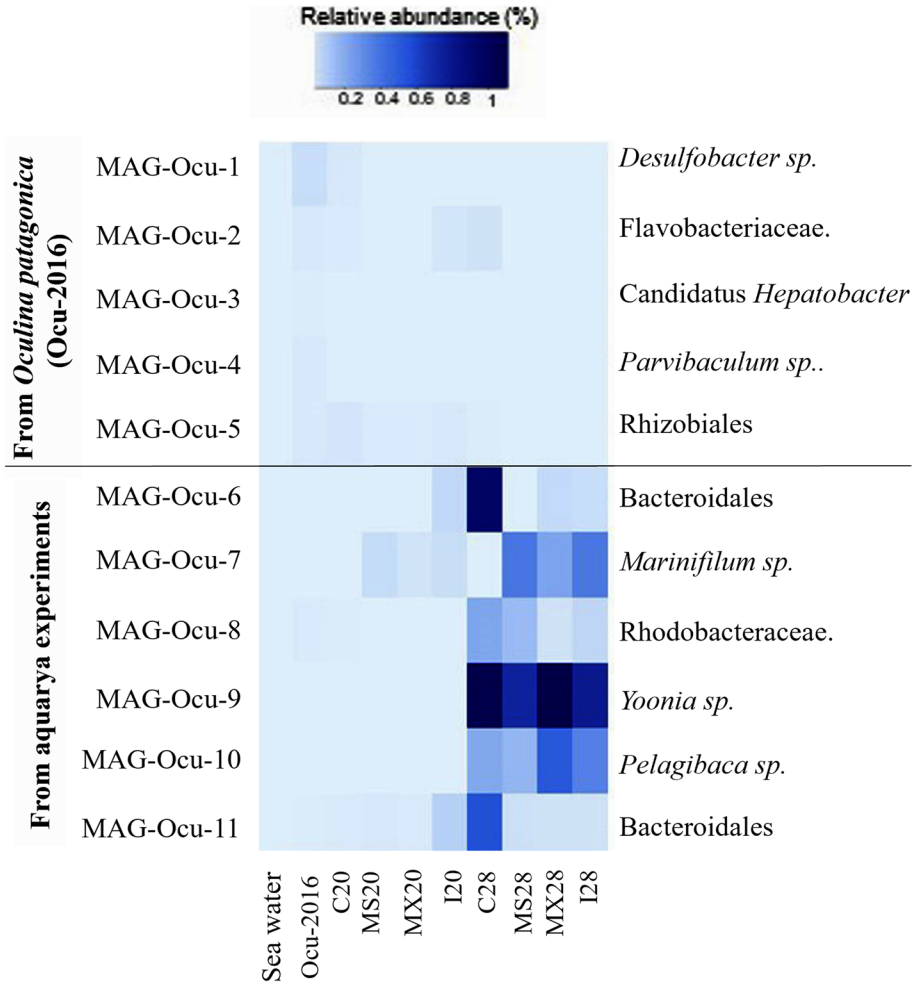

Fig. 6 Relative abundances of MAGs. The relative abundance of each MAG was estimated using fragment recruitment analyses carried out by BLASTn comparisons. Only reads that matched with over 95% identity and 70% coverage were considered. The fraction of nucleotides mapping to the respective MAG was normalized by the length of that MAG and size of the metagenome

an orthologue gene encoding the DddL enzyme, responsible for DMSP catabolism, was detected in a *Yoonia*-related MAG, with a more pronounced increase in coral samples maintained at 28 °C (Fig. 6). This suggests that sulfur compounds produced in response to stressors, like confinement or thermal stress, may contribute to shaping coral-associated bacterial communities. This supports the hypothesis proposed by Raina et al. [96] that DMSP and DMS play a pivotal role in structuring coral-associated bacterial communities.

Other genera that increased under confinement conditions and particularly under heat stress were *Marinifilum*, a member of the Bacteroidetes genus, and *Halodesulfovibrio*, originally affiliated with *Desulfovibrio* genus (Fig. 4). These genera have previously been observed in coral samples, and their proliferation under aquarium conditions has been documented by [97]. *Marinifilum* may play a role in the sulfur and carbon cycle, as well as lipid catabolism. On the other hand, *Halodesulfovibrio* has been identified as a secondary pathogen responsible for initiating and progressing black band disease in coral hosts, producing sulfide as a product of dissimilatory sulfate reduction [98].

The increase of bacterial bloomers under heat stress may be partially influenced by the recycling of metabolic waste products within the holobiont [99] and subsequent increase of available nutrients. Additionally, corals regularly release mucus into the surrounding seawater, which carries elevated concentrations of nutrients like organic carbon [100]. In heat-stressed corals, an increase in carbohydrate content in mucus has been detected [89], thus serving as a carbon source for heterotrophic microbes. To explore this idea, variation in the abundance of genes encoding carbohydrate-active enzymes (CAZymes) was examined. Compared to Ocu-2016, there was an increase in the proportion of glycosylhydrolases, glycosyltransferases, and carbohydrate-binding modules in corals maintained under experimental conditions, particularly in the thermally stressed ones (Supplementary Fig. 3). This suggests that mucus released under stressful conditions could provide newly accessible nutrients, serving as sustenance for fast-growing microbes. This process could potentially be enhanced by the decrease of other bacterial taxa and microeukaryotes under stress conditions, which may act as microbial regulators within the coral holobiont, as explained below.

Regarding viruses, it is well established that virus-like particles (VLPs) increase in corals during stressful conditions or bleaching events [85, 101]. In this case, recruitment analysis showed that one of the viruses, identified as Phycodnaviridae in the Ocu-2016 metagenome, clearly increased in abundance under thermal stress (Supplementary Fig. 4) when *Symbiodinium* abundances decreased. This fact suggests that this virus could be involved in the destruction of algal symbionts or the dysfunction of symbiont–host mutualism as mentioned above.

O. patagonica holobiont changes due to *Vibrio* infection under experimental conditions

All the above-described changes observed in confined or heat stress were enhanced in corals exposed to *Vibrio* cells (*V. coralliilyticus* and *V. mediterranei*), either directly or indirectly. Specifically, corals at 28 °C in the presence of *Vibrio* pathogens experienced a more significant increase in *Vibrio* species abundance compared to controls. Accordingly, a previous transcriptomic study carried out with these samples [34] revealed that pathogenic *Vibrio* release *quorum sensing* molecules, triggering alterations in coral-associated bacteria and an increase in other potential pathogens already present in the coral sample, thus explaining the increase of *Vibrio* spp. detected here. Furthermore, the increase in genera related to DMSP metabolism, such as *Marivitia*, *Ruegeria*, *Loktanella*, and *Yoonia*, was also more pronounced under the presence of *Vibrio* pathogens, which also suggests vibrios play a key role in coral microbiome modulation (Figs. 4 and 6).

Analysis of the viral contigs in these coral metagenomes revealed a significant decrease in the proportion of genomes classified as prophages in corals experiencing heat stress (0.002–0.0004%) and in those exposed to *Vibrio* coral pathogens (0.001–0.0005%) compared to the natural sample (0.03%) (Fig. 7A). To delve into the lysogenic dynamics within the coral holobiont, recruitment analyses were performed using only prophage genomes identified within bacterial contigs; this approach confirmed their integration into their respective bacterial hosts. Our data unveiled the induction of three distinct prophages in corals subjected to *Vibrio* infection (I20) or heat-induced stress (C28), and two of these prophages were found to be integrated within contigs belonging to the Rhodobacterales order (see Fig. 7B). These findings align well with recent research suggesting that coral pathogens, such as *V. coralliilyticus*, produce hydrogen peroxide to initiate the lytic cycle of prophages in their competitors, thereby providing the coral pathogen with an advantage by reducing competition during coral colonization [102]. This mechanism further contributes to host dysbiosis by shifting the balance from symbionts to pathobionts as observed in the human gut [103].

Conclusions

Taxonomic characterization of the *O. patagonica* holobiont showed a highly diverse microeukaryotic community. This, in addition to the well-known *Symbiodinium*,

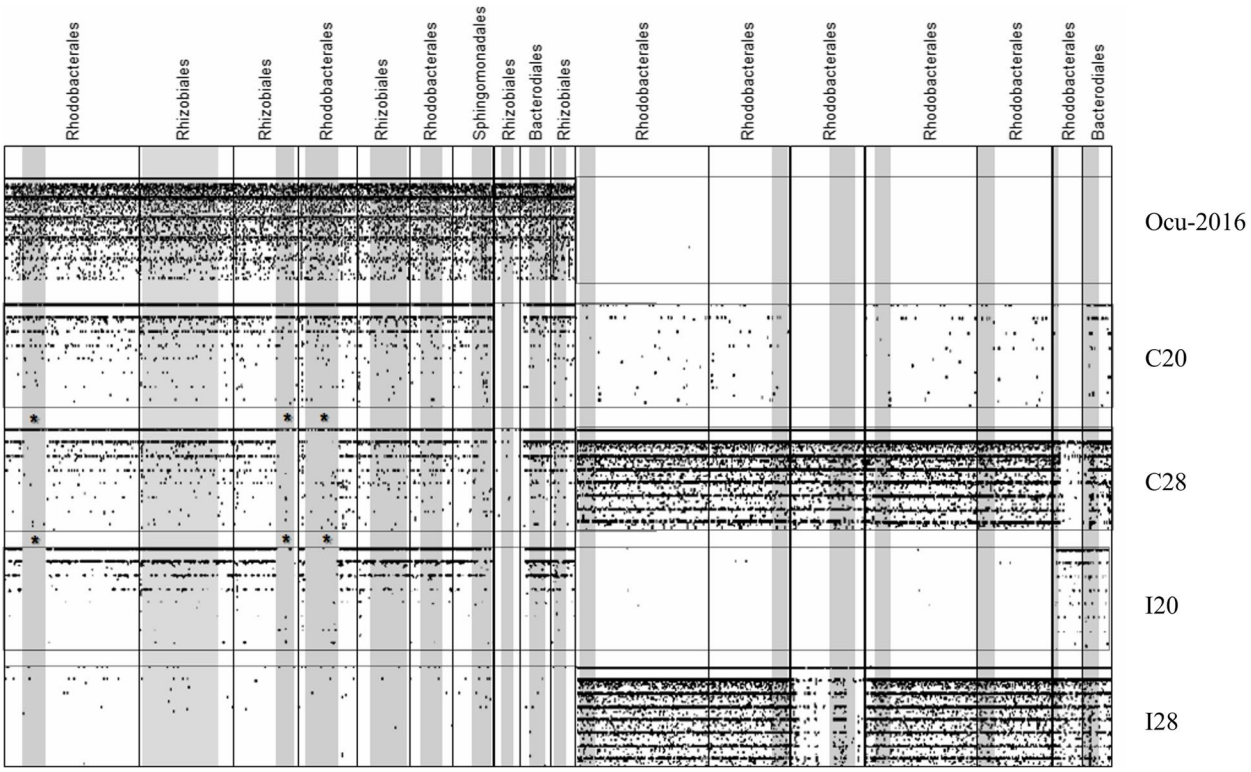

Fig. 7 Fragment recruitment plots for metagenome sequence reads on bacterial contigs with prophages (marked in grey in each contig) from each coral. The vertical axis indicates the sequence identity of an alignment between a metagenomic sequence and the reference contig using BLASTn, the identity ranges from 100% (top) to 95% (bottom). *Prophages induced from bacterial host upon heat or *Vibrio* infection stress

includes fungi from the genera *Aspergillus*, *Fusarium*, and *Rhizophagus*. Among prokaryotes, the most abundant genera found were the archaea *Nitrosopumilus* together with the bacteria *Streptomyces*, *Pseudomonas*, and *Bacillus*.

Our aquaria experiments were designed to address changes on coral microbiome related to different stressors. Importantly, many of these microeukaryotic symbionts suffered a significant decline (below detectable levels) in *O. patagonica*, particularly under heat stress. Concurrently, a notable increase in the abundance of the prokaryotic community was observed, shedding light on the complex interplay between these two communities. Our data suggest that the microeukaryotic community potentially exerts influence on the prokaryotic community dynamics, possibly through predation or the production of secondary metabolites with anti-bacterial activity.

The increase in the contribution of the prokaryotic community to the holobiont appears to coincide with an increase of CAZymes and genes responsible for DMSP catabolism. This correlation suggests the existence of a potential recycling mechanism for organic products generated by the holobiont under stress conditions. Thus, the alteration of conditions (such as temperature, confinement, and pathogen presence) within the coral holobiont appears to enhance the production of enzymes dedicated to carbohydrate degradation. This discovery underscores the dynamic and adaptive nature of microbial communities within coral ecosystems and offers exciting prospects for further research into the exploitation of these enzymatic resources in various industrial and environmental contexts.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40168-024-01921-x.

Additional file 1: Scripts used for the statistical analysis.

Additional file 2: Supplementary Tables 1–8 and Supplementary Figs. 1–4.

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Authors' contributions
E.R.-P. and J.A. conceived and designed the study. E.R.-P conducted experiments. A.B.M.-C, F.R. and E.R.-P. analyzed the metagenomic data. A.B.M.-C,

E.R.-P, F.R. and J.A wrote the manuscript, and all of us contributed substantially with discussion.

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Data availability
The raw sequences of the wild-type *O. patagonica* metagenome datasets (Ocu-2016) have been deposited in the SRA repository under the BioProject number PRJNA661426. Metagenomes from the infection experiments and their respective controls can be found in the [34] (PRJNA612159).

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. LaJeunesse TC, Parkinson JE, Gabrielson PW, Jeong HJ, Reimer JD, Voolstra CR, et al. Systematic revision of symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. Curr Biol. 2018;28(16):2570–80 e6. https://doi.org/10.1016/j.cub.2018.07.008.
2. Knowlton N, Rohwer F. Multispecies microbial mutualisms on coral reefs: the host as a habitat. Am Nat. 2003;162(4 Suppl):S51–62.
3. Rohwer FSV, Azam F, Knowlton N. Diversity and distribution of coral-associated bacteria. Mar Ecol Prog Ser. 2002;243:1–10. https://doi.org/10.3354/meps243001.
4. van Oppen MJH, Blackall LL. Coral microbiome dynamics, functions and design in a changing world. Nat Rev Microbiol. 2019;17(9):557–67. https://doi.org/10.1038/s41579-019-0223-4.
5. Muscatine L, Falkowski PG, Porter JW, Dubinsky Z. Fate of photosynthetic fixed carbon in light- and shade-adapted colonies of the symbiotic coral Stylophora pistillata. Proceed Royal Soc London Series B Biol Sci. 1984;222(1227):181–202.
6. Voolstra CR, Suggett DJ, Peixoto RS, Parkinson JE, Quigley KM, Silveira CB, Sweet M, Muller EM, Barshis DJ, Bourne DG, Aranda M. Extending the natural adaptive capacity of coral holobionts. Nat Rev Earth Environ. 2021;2:747–62.
7. Fine M, Loya Y. Endolithic algae: an alternative source of photoassimilates during coral bleaching. Proc Biol Sci. 2002;269(1497):1205–10. https://doi.org/10.1098/rspb.2002.1983.
8. Schlichter D, Zscharnack B, Krisch H. Transfer of photoassimilates from endolithic algae to coral tissue. Naturwissenschaften. 1995;82(12):561–4. https://doi.org/10.1007/Bf01140246.
9. Sangsawang L, Casareto BE, Ohba H, Vu HM, Meekaew A, Suzuki T, et al. (13)C and (15)N assimilation and organic matter translocation by the endolithic community in the massive coral Porites lutea. R Soc Open Sci. 2017;4(12):171201. https://doi.org/10.1098/rsos.171201.
10. Littman RA, Willis BL, Pfeffer C, Bourne DG. Diversities of coral-associated bacteria differ with location, but not species, for three acroporid corals on the Great Barrier Reef. FEMS Microbiol Ecol. 2009;68(2):152–63.
11. Bourne D, Iida Y, Uthicke S, Smith-Keune C. Changes in coral-associated microbial communities during a bleaching event. ISME J. 2008;2(4):350–63. https://doi.org/10.1038/ismej.2007.112.
12. Zhou Z, Zhao S, Tang J, Liu Z, Wu Y, Wang Y, et al. Altered immune landscape and disrupted coral-Symbiodinium symbiosis in the scleractinian coral Pocillopora damicornis by Vibrio coralliilyticus challenge. Front Physiol. 2019;10:366. https://doi.org/10.3389/fphys.2019.00366.
13. Meron D, Rodolfo-Metalpa R, Cunning R, Baker AC, Fine M, Banin E. Changes in coral microbial communities in response to a natural pH gradient. ISME J. 2012;6(9):1775–85. https://doi.org/10.1038/ismej.2012.19.
14. Lee ST, Davy SK, Tang SL, Fan TY, Kench PS. Successive shifts in the microbial community of the surface mucus layer and tissues of the coral Acropora muricata under thermal stress. FEMS Microbiol Ecol. 2015;91(12):fiv142. https://doi.org/10.1093/femsec/fiv142.
15. Li J, Zhou Y, Qin Y, Wei J, Shigong P, Ma H, et al. Assessment of the juvenile vulnerability of symbiont-bearing giant clams to ocean acidification. Sci Total Environ. 2022;812:152265. https://doi.org/10.1016/j.scitotenv.2021.152265.
16. Klinges JG, Patel SH, Duke WC, Muller EM, Vega Thurber RL. Phosphate enrichment induces increased dominance of the parasite Aquarickettsia in the coral Acropora cervicornis. FEMS Microbiol Ecol. 2022;98(2):fiac013. https://doi.org/10.1093/femsec/fiac013.
17. Galand PE, Chapron L, Meistertzheim AL, Peru E, Lartaud F. The effect of captivity on the dynamics of active bacterial communities differs between two deep-sea coral species. Front Microbiol. 2018;9:2565. https://doi.org/10.3389/fmicb.2018.02565.
18. Kooperman N, Ben-Dov E, Kramarsky-Winter E, Barak Z, Kushmaro A. Coral mucus-associated bacterial communities from natural and aquarium environments. FEMS Microbiol Lett. 2007;276(1):106–13. https://doi.org/10.1111/j.1574-6968.2007.00921.x.
19. Calvo-Diaz A, Diaz-Perez L, Suarez LA, Moran XA, Teira E, Maranon E. Decrease in the autotrophic-to-heterotrophic biomass ratio of picoplankton in oligotrophic marine waters due to bottle enclosure. Appl Environ Microbiol. 2011;77(16):5739–46. https://doi.org/10.1128/AEM.00066-11.
20. Dinasquet J, Kragh T, Schroter ML, Sondergaard M, Riemann L. Functional and compositional succession of bacterioplankton in response to a gradient in bioavailable dissolved organic carbon. Environ Microbiol. 2013;15(9):2616–28. https://doi.org/10.1111/1462-2920.12178.
21. Massana R, Pedrós‐Alió C, Casamayor EO, Gasol JM. Changes in marine bacterioplankton phylogenetic composition during incubations designed to measure biogeochemically significant parameters. Limnol Oceanog. 2001;46:1181–8. https://doi.org/10.4319/lo.2001.46.5.1181.
22. Cai L, Tian RM, Zhou G, Tong H, Wong YH, Zhang W, et al. Exploring coral microbiome assemblages in the South China Sea. Sci Rep. 2018;8(1):2428.
23. Robbins SJ, Singleton CM, Chan CX, Messer LF, Geers AU, Ying H, et al. A genomic view of the reef-building coral Porites lutea and its microbial symbionts. Nat Microbiol. 2019;4(12):2090–100.
24. Weynberg KD, Wood-Charlson EM, Suttle CA, van Oppen MJ. Generating viral metagenomes from the coral holobiont. Front Microbiol. 2014;5:206. https://doi.org/10.3389/fmicb.2014.00206.
25. Rubio-Portillo E, Souza-Egipsy V, Ascaso C, de Los Rios Murillo A, Ramos-Espla AA, Anton J. Eukarya associated with the stony coral Oculina patagonica from the mediterranean sea. Mar Genomics. 2014;17:17–23. https://doi.org/10.1016/j.margen.2014.06.002.
26. Leydet KP, Grupstra CGB, Coma R, Ribes M, Hellberg ME. Host-targeted RAD-Seq reveals genetic changes in the coral Oculina patagonica associated with range expansion along the Spanish Mediterranean coast. Mol Ecol. 2018;27(11):2529–43. https://doi.org/10.1111/mec.14702.
27. Serrano E, Ribes M, Coma R. Demographics of the zooxanthellate coral Oculina patagonica along the Mediterranean Iberian coast in relation to environmental parameters. Sci Total Environ. 2018;634:1580–92. https://doi.org/10.1016/j.scitotenv.2018.04.032.
28. Rubio-Portillo E, Yarza P, Penalver C, Ramos-Espla AA, Anton J. New insights into Oculina patagonica coral diseases and their associated Vibrio spp. communities. ISME J. 2014;8(9):1794–807. https://doi.org/10.1038/ismej.2014.33.
29. Zaquin T, Zaslansky P, Pinkas I, Mass T. Simulating bleaching: long-term adaptation to the dark reveals phenotypic plasticity of the

Mediterranean sea coral Oculina patagonica. Front Marine Sci. 2019;6:662. https://doi.org/10.3389/fmars.2019.00662.
30. Rodolfo-Metalpa R, Hoogenboom MO, Rottier C, Ramos-Espla A, Baker AC, Fine M, et al. Thermally tolerant corals have limited capacity to acclimatize to future warming. Glob Chang Biol. 2014;20(10):3036–49. https://doi.org/10.1111/gcb.12571.
31. Rubio-Portillo E, Kersting DK, Linares C, Ramos-Espla AA, Anton J. Biogeographic differences in the microbiome and pathobiome of the coral Cladocora caespitosa in the Western Mediterranean Sea. Front Microbiol. 2018;9:22. https://doi.org/10.3389/fmicb.2018.00022.
32. Armoza-Zvuloni R, Segal R, Kramarsky-Winter E, Loya Y. Repeated bleaching events may result in high tolerance and notable gametogenesis in stony corals: Oculina patagonica as a model. Mar Ecol Prog Ser. 2011;426:149–59.
33. Shenkar N, Fine M, Loya Y. Size matters: bleaching dynamics of the coral Oculina patagonica. Marine Ecol Progr Ser. 2005;294:181–8.
34. Rubio-Portillo E, Martin-Cuadrado AB, Caraballo-Rodriguez AM, Rohwer F, Dorrestein PC, Anton J. Virulence as a side effect of interspecies interaction in Vibrio coral pathogens. mBio. 2020;11(4):e00201. https://doi.org/10.1128/mBio.00201-20.
35. Schmieder R, Edwards R. Quality control and preprocessing of metagenomic datasets. Bioinformatics. 2011;27(6):863–4. https://doi.org/10.1093/bioinformatics/btr026.
36. Rodriguez RL, Konstantinidis KT. Nonpareil: a redundancy-based approach to assess the level of coverage in metagenomic datasets. Bioinformatics. 2014;30(5):629–35. https://doi.org/10.1093/bioinformatics/btt584.
37. Menzel P, Ng KL, Krogh A. Fast and sensitive taxonomic classification for metagenomics with Kaiju. Nat Commun. 2016;7:11257. https://doi.org/10.1038/ncomms11257.
38. Li D, Luo R, Liu CM, Leung CM, Ting HF, Sadakane K, et al. MEGAHIT v1.0: a fast and scalable metagenome assembler driven by advanced methodologies and community practices. Methods. 2016;102:3–11. https://doi.org/10.1016/j.ymeth.2016.02.020.
39. Nurk S, Meleshko D, Korobeynikov A, Pevzner PA. metaSPAdes: a new versatile metagenomic assembler. Genome Res. 2017;27(5):824–34. https://doi.org/10.1101/gr.213959.116.
40. Kang DD, Froula J, Egan R, Wang Z. MetaBAT, an efficient tool for accurately reconstructing single genomes from complex microbial communities. PeerJ. 2015;3: e1165. https://doi.org/10.7717/peerj.1165.
41. Wu YW, Tang YH, Tringe SG, Simmons BA, Singer SW. MaxBin: an automated binning method to recover individual genomes from metagenomes using an expectation-maximization algorithm. Microbiome. 2014;2:26. https://doi.org/10.1186/2049-2618-2-26.
42. Sieber CMK, Probst AJ, Sharrar A, Thomas BC, Hess M, Tringe SG, et al. Recovery of genomes from metagenomes via a dereplication, aggregation and scoring strategy. Nat Microbiol. 2018;3(7):836–43. https://doi.org/10.1038/s41564-018-0171-1.
43. Eren AM, Esen OC, Quince C, Vineis JH, Morrison HG, Sogin ML, et al. Anvi'o: an advanced analysis and visualization platform for 'omics data. PeerJ. 2015;3:e1319. https://doi.org/10.7717/peerj.1319.
44. Konstantinidis KT, Rossello-Mora R, Amann R. Uncultivated microbes in need of their own taxonomy. ISME J. 2017;11(11):2399–406. https://doi.org/10.1038/ismej.2017.113.
45. Ondov BD, Treangen TJ, Melsted P, Mallonee AB, Bergman NH, Koren S, et al. Mash: fast genome and metagenome distance estimation using MinHash. Genome Biol. 2016;17(1):132. https://doi.org/10.1186/s13059-016-0997-x.
46. Chaumeil PA, Mussig AJ, Hugenholtz P, Parks DH. GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. Bioinformatics. 2019;36(6):1925–7. https://doi.org/10.1093/bioinformatics/btz848.
47. Hyatt D, Chen GL, Locascio PF, Land ML, Larimer FW, Hauser LJ. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics. 2010;11:119. https://doi.org/10.1186/1471-2105-11-119.
48. Huerta-Cepas J, Szklarczyk D, Heller D, Hernandez-Plaza A, Forslund SK, Cook H, et al. eggNOG 5.0: a hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. Nucleic Acids Res. 2019;47(D1):D309–14. https://doi.org/10.1093/nar/gky1085.
49. Bateman A, Coin L, Durbin R, Finn RD, Hollich V, Griffiths-Jones S, et al. The Pfam protein families database. Nucleic Acids Res. 2004;32(Database issue):D138–41. https://doi.org/10.1093/nar/gkh121.
50. Tatusov RL, Natale DA, Garkavtsev IV, Tatusova TA, Shankavaram UT, Rao BS, et al. The COG database: new developments in phylogenetic classification of proteins from complete genomes. Nucleic Acids Res. 2001;29(1):22–8. https://doi.org/10.1093/nar/29.1.22.
51. Haft DH, Selengut JD, Richter RA, Harkins D, Basu MK, Beck E. TIGRFAMs and genome properties in 2013. Nucleic Acids Res. 2013;41(Database issue):D387–95. https://doi.org/10.1093/nar/gks1234.
52. Kanehisa M, Sato Y, Morishima K. BlastKOALA and GhostKOALA: KEGG tools for functional characterization of genome and metagenome sequences. J Mol Biol. 2016;428(4):726–31. https://doi.org/10.1016/j.jmb.2015.11.006.
53. Medema MH, Blin K, Cimermancic P, de Jager V, Zakrzewski P, Fischbach MA, et al. antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. Nucleic Acids Res. 2011;39(Web Server issue):W339–46. https://doi.org/10.1093/nar/gkr466.
54. Zhang H, Yohe T, Huang L, Entwistle S, Wu P, Yang Z, et al. dbCAN2: a meta server for automated carbohydrate-active enzyme annotation. Nucleic Acids Res. 2018;46(W1):W95–101. https://doi.org/10.1093/nar/gky418.
55. Bushnell B. 2014. BBMap: a fast, accurate, splice-aware aligner Tech. Rep. LBNL-7065E Lawrence Berkeley Natl. Lab. Berkeley, CA. https://sourceforge.net/projects/bbmap/.
56. Rodriguez RL, Tsementzi D, Luo C, Konstantinidis KT. Iterative subtractive binning of freshwater chronoseries metagenomes identifies over 400 novel species and their ecologic preferences. Environ Microbiol. 2020;22(8):3394–412. https://doi.org/10.1111/1462-2920.15112.
57. Nayfach S, Pollard KS. Average genome size estimation improves comparative metagenomics and sheds light on the functional ecology of the human microbiome. Genome Biol. 2015;16(1):51. https://doi.org/10.1186/s13059-015-0611-7.
58. Ulyantsev VI, Kazakov SV, Dubinkina VB, Tyakht AV, Alexeev DG. MetaFast: fast reference-free graph-based comparison of shotgun metagenomic data. Bioinformatics. 2016;32(18):2760–7.
59. Sunagawa S, Coelho LP, Chaffron S, Kultima JR, Labadie K, Salazar G, et al. Ocean plankton. Structure and function of the global ocean microbiome. Science. 2015;348(6237):1261359. https://doi.org/10.1126/science.1261359.
60. Haro-Moreno JM, Lopez-Perez M, de la Torre JR, Picazo A, Camacho A, Rodriguez-Valera F. Fine metagenomic profile of the Mediterranean stratified and mixed water columns revealed by assembly and recruitment. Microbiome. 2018;6(1):128.
61. Roux S, Enault F, Hurwitz BL, Sullivan MB. VirSorter: mining viral signal from microbial genomic data. PeerJ. 2015;3:e985. https://doi.org/10.7717/peerj.985.
62. Arndt D, Grant JR, Marcu A, Sajed T, Pon A, Liang Y, et al. PHASTER: a better, faster version of the PHAST phage search tool. Nucleic Acids Res. 2016;44(W1):W16–21. https://doi.org/10.1093/nar/gkw387.
63. Fu L, Niu B, Zhu Z, Wu S, Li W. CD-HIT: accelerated for clustering the next-generation sequencing data. Bioinformatics. 2012;28(23):3150–2. https://doi.org/10.1093/bioinformatics/bts565.
64. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13(11):2498–504.
65. Good PI. Introduction to statistics through resampling methods and R/S-PLUS. Wiley; 2012.
66. Thornhill DJ, Howells EJ, Wham DC, Steury TD, Santos SR. Population genetics of reef coral endosymbionts (Symbiodinium, Dinophyceae). Mol Ecol. 2017;26(10):2640–59. https://doi.org/10.1111/mec.14055.
67. Kramarsky-Winter E, Harel M, Sibon N, Dov EB, Brickner I, Loya Y, Kushmaro A. Identification of a protist-coral association and its possible ecological role. Marine Ecol Progr Ser. 2006;317:67–73. https://doi.org/10.3354/meps317067.
68. Kohler S, Delwiche CF, Denny PW, Tilney LG, Webster P, Wilson RJ, et al. A plastid of probable green algal origin in Apicomplexan parasites. Science. 1997;275(5305):1485–9. https://doi.org/10.1126/science.275.5305.1485.

69. Janouskovec J, Horak A, Barott KL, Rohwer FL, Keeling PJ. Global analysis of plastid diversity reveals apicomplexan-related lineages in coral reefs. Curr Biol. 2012;22(13):R518–9.
70. Janouskovec J, Horak A, Barott KL, Rohwer FL, Keeling PJ. Environmental distribution of coral-associated relatives of apicomplexan parasites. Isme J. 2013;7(2):444–7.
71. Bentis CJ, Kaufman L, Golubic S. Endolithic fungi in reef-building corals (Order : Scleractinia) are common, cosmopolitan, and potentially pathogenic. Biol Bull. 2000;198(2):254–60.
72. Raghukumar C, Ravindran J. Fungi and their role in corals and coral reef ecosystems. Prog Mol Subcell Biol. 2012;53:89–113.
73. Litchman E. Invisible invaders: non-pathogenic invasive microbes in aquatic and terrestrial ecosystems. Ecol Lett. 2010;13(12):1560–72. https://doi.org/10.1111/j.1461-0248.2010.01544.x.
74. Yarden O, Ainsworth TD, Roff G, Leggat W, Fine M, Hoegh-Guldberg O. Increased prevalence of ubiquitous ascomycetes in an acropoid coral (Acropora formosa) exhibiting symptoms of Brown Band syndrome and skeletal eroding band disease. Appl Environ Microbiol. 2007;73(8):2755–7. https://doi.org/10.1128/AEM.02738-06.
75. Savary R, Masclaux FG, Wyss T, Droh G, Cruz Corella J, Machado AP, et al. A population genomics approach shows widespread geographical distribution of cryptic genomic forms of the symbiotic fungus Rhizophagus irregularis. Isme J. 2018;12(1):17–30.
76. Siboni N, Ben-Dov E, Sivan A, Kushmaro A. Global distribution and diversity of coral-associated Archaea and their possible role in the coral holobiont nitrogen cycle. Environ Microbiol. 2008;10(11):2979–90. https://doi.org/10.1111/j.1462-2920.2008.01718.x.
77. Rubio-Portillo E, Santos F, Martinez-Garcia M, de Los RA, Ascaso C, Souza-Egipsy V, et al. Structure and temporal dynamics of the bacterial communities associated to microhabitats of the coral Oculina patagonica. Environ Microbiol. 2016;18(12):4564–78. https://doi.org/10.1111/1462-2920.13548.
78. McCutcheon JP, Moran NA. Extreme genome reduction in symbiotic bacteria. Nat Rev Microbiol. 2011;10(1):13–26. https://doi.org/10.1038/nrmicro2670.
79. Hochart C, Paoli L, Ruscheweyh HJ, Salazar G, Boissin E, Romac S, et al. Ecology of Endozoicomonadaceae in three coral genera across the Pacific Ocean. Nat Commun. 2023;14(1):3037. https://doi.org/10.1038/s41467-023-38502-9.
80. Tang YZ, Koch F, Gobler CJ. Most harmful algal bloom species are vitamin B1 and B12 auxotrophs. Proc Natl Acad Sci U S A. 2010;107(48):20756–61. https://doi.org/10.1073/pnas.1009566107.
81. Al-Khodor S, Price CT, Kalia A, Abu KY. Functional diversity of ankyrin repeats in microbial proteins. Trends Microbiol. 2010;18(3):132–9. https://doi.org/10.1016/j.tim.2009.11.004.
82. Jernigan KK, Bordenstein SR. Ankyrin domains across the Tree of Life. PeerJ. 2014;2:e264. https://doi.org/10.7717/peerj.264.
83. Lesser MP, Falcon LI, Rodriguez-Roman A, Enriquez S, Hoegh-Guldberg O, Iglesias-Prieto R. Nitrogen fixation by symbiotic cyanobacteria provides a source of nitrogen for the scleractinian coral Montastraea cavernosa. Mar Ecol-Prog Ser. 2007;346:143–52. https://doi.org/10.3354/meps07008.
84. Beaulaurier J, Luo E, Eppley JM, Uyl PD, Dai X, Burger A, et al. Assembly-free single-molecule sequencing recovers complete virus genomes from natural microbial communities. Genome Res. 2020;30(3):437–46. https://doi.org/10.1101/gr.251686.119.
85. Correa AM, Ainsworth TD, Rosales SM, Thurber AR, Butler CR, Vega Thurber RL. Viral outbreak in corals associated with an in situ bleaching event: atypical herpes-like viruses and a new megavirus infecting Symbiodinium. Front Microbiol. 2021;7:127.
86. Lawrence SA, Wilson WH, Davy JE, Davy SK. Latent virus-like infections are present in a diverse range of Symbiodinium spp. (Dinophyta). J Phycol. 2014;50(6):984–97. https://doi.org/10.1111/jpy.12242.
87. Correa AM, Welsh RM, Vega Thurber RL. Unique nucleocytoplasmic dsDNA and +ssRNA viruses are associated with the dinoflagellate endosymbionts of corals. ISME J. 2013;7(1):13–27. https://doi.org/10.1038/ismej.2012.75.
88. Haro-Moreno JM, Rodriguez-Valera F, Lopez-Perez M. Prokaryotic population dynamics and viral predation in a marine succession experiment using metagenomics. Front Microbiol. 2019;10:2926. https://doi.org/10.3389/fmicb.2019.02926.
89. Wright RM, Strader ME, Genuise HM, Matz M. Effects of thermal stress on amount, composition, and antibacterial properties of coral mucus. PeerJ. 2019;7:e6849.
90. Xu JH, Lai KH, Su YD, Chang YC, Peng BR, Backlund A, et al. Briaviolides K-N, New briarane-type diterpenoids from cultured octocoral Briareum violaceum. Mar Drugs. 2018;16(3):75. https://doi.org/10.3390/md16030075.
91. Abd El-Rahman TMA, Tharwat NA, Abo El-Souad SMS, El-Beih AA, El-Diwany AI. Biological activities and variation of symbiotic fungi isolated from coral reefs collected from Red Sea in Egypt. Mycology. 2020;11(3):243–55. https://doi.org/10.1080/21501203.2020.1741470.
92. Pootakham W, Mhuantong W, Yoocha T, Putchim L, Jomchai N, Sonthirod C, et al. Heat-induced shift in coral microbiome reveals several members of the Rhodobacteraceae family as indicator species for thermal stress in Porites lutea. Microbiologyopen. 2019;8(12):e935. https://doi.org/10.1002/mbo3.935.
93. Raina JB, Tapiolas DM, Foret S, Lutz A, Abrego D, Ceh J, et al. DMSP biosynthesis by an animal and its role in coral thermal stress response. Nature. 2013;502(7473):677–80. https://doi.org/10.1038/nature12677.
94. Gardner SG, Raina JB, Ralph PJ, Petrou K. Reactive oxygen species (ROS) and dimethylated sulphur compounds in coral explants under acute thermal stress. J Exp Biol. 2017;220(Pt 10):1787–91. https://doi.org/10.1242/jeb.153049.
95. Gardner SG, Nitschke MR, O'Brien J, Motti CA, Seymour JR, Ralph PJ, Petrou K, Raina JB. Increased DMSP availability during thermal stress influences DMSP-degrading bacteria in coral mucus. Front Marine Sci. 2022;9:912862. https://doi.org/10.3389/fmars.2022.912862.
96. Raina JB, Dinsdale EA, Willis BL, Bourne DG. Do the organic sulfur compounds DMSP and DMS drive coral microbial associations? Trends Microbiol. 2010;18(3):101–8. https://doi.org/10.1016/j.tim.2009.12.002.
97. Zhang Y, Yang Q, Zhang Y, Ahmad M, Ling J, Tang X, et al. Shifts in abundance and network complexity of coral bacteria in response to elevated ammonium stress. Sci Total Environ. 2021;768:144631. https://doi.org/10.1016/j.scitotenv.2020.144631.
98. Brownell AC, Richardson LL. Sulfate reducing bacteria as secondary and necessary pathogens in black band disease of corals. Rev Biol Trop. 2014;62:62.
99. Aranda M, Li Y, Liew YJ, Baumgarten S, Simakov O, Wilson MC, et al. Genomes of coral dinoflagellate symbionts highlight evolutionary adaptations conducive to a symbiotic lifestyle. Sci Rep. 2016;6:39734. https://doi.org/10.1038/srep39734.
100. Nakajima Y, Nishikawa A, Isomura N, Iguchi A, Sakai K. Genetic connectivity in the broadcast-spawning coral Acropora digitifera analyzed by microsatellite markers on the Sekisei Reef, southwestern Japan. Zoolog Sci. 2009;26(3):209–15. https://doi.org/10.2108/zsj.26.209.
101. Vega Thurber RL, Barott KL, Hall D, Liu H, Rodriguez-Mueller B, Desnues C, et al. Metagenomic analysis indicates that stressors induce production of herpes-like viruses in the coral Porites compressa. Proc Natl Acad Sci U S A. 2008;105(47):18413–8. https://doi.org/10.1073/pnas.0808985105.
102. Wang W, Tang K, Wang P, Zeng Z, Xu T, Zhan W, et al. The coral pathogen Vibrio coralliilyticus kills non-pathogenic holobiont competitors by triggering prophage induction. Nat Ecol Evol. 2022;6(8):1132–44. https://doi.org/10.1038/s41559-022-01795-y.
103. Mills S, Shanahan F, Stanton C, Hill C, Coffey A, Ross RP. Movers and shakers: influence of bacteriophages in shaping the mammalian gut microbiota. Gut Microbes. 2013;4(1):4–16. https://doi.org/10.4161/gmic.22371.

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