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Microbial and transcriptional response of *Acropora valida* and *Turbinaria peltata* to *Vibrio coralliilyticus* challenge: insights into corals disease resistance

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Abstract

Background Coral diseases are significant drivers of global coral reef degradation, with pathogens dominated by *Vibrio coralliilyticus* playing a prominent role in the development of coral diseases. Coral phenotype, symbiotic microbial communities, and host transcriptional regulation have been well-established as factors involved in determining coral disease resistance, but the underlying mechanisms remain incompletely understood.

Methods This study employs high-throughput sequencing to analyse the symbiotic microbial and transcriptional response of the hosts in order to evaluate the disease resistance of *Acropora valida* and *Turbinaria peltata* exposed to *Vibrio coralliilyticus*.

Results *A. valida* exhibited pronounced bleaching and tissue loss within 7 h of pathogen infection, whereas *T. peltata* showed no signs of disease throughout the experiment. Microbial diversity analyses revealed that *T. peltata* had a more flexible microbial community and a higher relative abundance of potential beneficial bacteria compared to *A. valida*. Although *Vibrio* inoculation resulted in a more significant decrease in the Symbiodiniaceae density of *A. valida* compared to that of *T. peltata*, it did not lead to recombination of the coral host and Symbiodiniaceae in either coral species. RNA-seq analysis revealed that the interspecific differences in the transcriptional regulation of hosts after *Vibrio* inoculation. Differentially expressed genes in *A. valida* were mainly enriched in the pathways associated with energy supply and immune response, such as G protein-coupled receptor signaling, toll-like receptor signaling, regulation of TOR signaling, while these genes in *T. peltata* were mainly involved in the pathway related to immune homeostasis and ion transport, such as JAK-STAT signaling pathway and regulation of ion transport.

Conclusions Pathogenic challenges elicit different microbial and transcriptional shifts across coral species. This study offers novel insights into molecular mechanisms of coral resistance to disease.

Keywords Coral holobionts, *Vibrio coralliilyticus*, Microbial community, Transcriptome, Disease resistance

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Background

Coral reefs are essential constituents of marine ecosystems with high biodiversity and primary productivity, however, they confront numerous survival pressures [1]. Coral diseases have emerged as a pivotal driver of global coral reef degradation [2], which have caused large-scale and severe degradation of many coral reefs [3]. The threat of disease-induced extinction looms over numerous coral species [4]. Over the last three decades, coral reefs worldwide have experienced a substantial decline of approximately 30% due to new coral diseases, which is anticipated to intensify with ongoing deterioration of the marine environment [5]. Bacterial pathogens, especially *Vibrio*-related members, significantly contribute to a diverse spectrum of coral diseases [6], encompassing white band disease, yellow band disease, white syndrome, etc. [7]. *V. coralliilyticus* was initially identified in diseased and bleached *Pocillopora damicornis* in the Indian Ocean, which has the potential to induce disease in various corals, such as *P. damicornis* [8], *Montipora capitata* [9], and soft corals [8]. Moreover, it has been linked to outbreaks of white syndrome in several coral reef [10]. *Vibrio* targets the host through signaling compounds, such as dimethylsulfoniopropionate (DMSP) released by the host, subsequently disrupts the symbiotic relationship between the host and Symbiodiniaceae, resulting in a substantial loss of Symbiodiniaceae over a short time [7]. Additionally, *V. coralliilyticus* produces zinc-metalloprotease, which contributes to coral tissue lesions and coral bleaching. The pathogenesis and genetic information of *V. coralliilyticus* have been elucidated [8], establishing it as a model pathogen of coral diseases research. Nevertheless, it is important to note that interspecific variations in disease resistance exist among diverse coral species. Generally, corals with faster growth rates and thinner tissues seem more susceptible [11]. Previous studies have frequently reported the susceptibility of branching corals such as *Pocillopora*, *Acropora*, and *Montipora* to pathogenic *Vibrio,* whereas this characteristic has rarely been reported in massive corals such as *Favites* and *Turbinaria* [7, 12, 13]. The pathogen of white band disease is considered to be exclusively pathogenic for *Acropora* [14], while white plague disease type II can affect dozens of coral species [15]. Furthermore, *Montastraea cavernosa*, *Porites porites*, and *Porites astreoides* have generally exhibit high disease resistance to white plague, but their resistance did not seem to be effective against stony coral tissue loss disease [16]. While interspecific differences in disease susceptibility among corals have been mentioned in numerous studies, the mechanism underlying interspecific differences in coral disease resistance remains unclear.

The coral holobiont constitutes a complex and diverse system wherein homeostasis is preserved through the collaboration of the host and its associated symbiotic microorganisms, encompassing Symbiodiniaceae, bacteria, fungi, archaea, endophytic algae, protists and viruses. Collectively, they establish a stable mutualistic symbiotic relationship and facilitate crucial material cycle processes, thereby providing a standfast foundation for the homeostasis and vitality of coral holobiont [1, 17]. Furthermore, specific symbiotic bacteria act as natural protective barrier by synthesizing antimicrobial agents [17–19]. Therefore, the equilibrium of microbial communities emerges as pivotal for the homeostasis and functional integrity of coral holobiont. Nevertheless, coral symbiotic microbial communities are highly sensitive to environmental changes, especially disease and potential pathogens. A considerable number of studies have reported the community dynamics exposed to the disease stress [20–25], but the differential patterns of community variation have gradually attracted attention recently. Certain studies identify an increase in bacterial α-diversity as a result of disease stress [20, 24, 25], while the opposite pattern of change in α-diversity has been found in other studies [22, 23], and the differential pattern of change in β-diversity was also observed [23, 26]. It has been hypothesized that microbial community stability could confer greater resistance to environmental challenges [27, 28], but recent research has found that corals that exhibit strong bacterial community dynamics appear to possess higher disease resistance [29]. According to the Coral Probiotic Hypothesis [30], such dynamics may empower corals to selectively shape microbial community assemblages that favour the coral holobiont in various environmental conditions, thus enhance the resilience and adaptability [5]. While dissecting the details of coral disease resistance through the dynamics of bacterial communities is essential, it is not sufficient to explain the underlying mechanisms of coral disease resistance, as Symbiodiniaceae with high abundance and diversity inhabit in the holobiont, which drive the energy transfer of the holobiont [17]. Although the association between the availability of organic nutrients and holobiont resistance and resilience has been emphasized [31, 32], little attention has been focused on the association between the Symbiodiniaceae dynamics and host disease resistance. We propose that different symbiotic microorganisms play different roles in holobiont responses to disease stress, and that exploring their dynamics could enrich our understanding of coral disease resistance.

Coral innate immune process involves the recognition of pathogens through mannose-binding lectin, toll-like receptors, and the elimination of pathogens through the complement system, apoptosis, and autophagy [33].

Research on corals exposed to lipopolysaccharides revealed that disease-susceptible corals demonstrated a more pronounced up-regulation of apoptosis, while disease-resistant corals uniquely activated autophagy [34]. Additionally, transcriptomic studies have identified the primary pathways associated with innate immunity in corals, encompassing cellular immunity, the prophenoloxidase-activated melanization response, and basic oxidative pathways [35]. It was observed that *V. coralliilyticus* stimulates the immune response of corals at ambient temperature (25℃), leading to the up-regulation of genes related to prophenoloxidase activating enzyme and laccase [35]. However, at high temperature (26–32℃), *V. coralliilyticus* infection significant suppressed host immune activity due to the temperature dependence of *V. coralliilyticus*, leading a significant down-regulation of immune-related genes such P-selection-like protein and antimicrobial peptide gene [36, 37]. Despite the distinct roles played by the coral symbiotic microbes and host innate immunity in shaping coral disease resistance, there is limited knowledge concerning the interconnections among microbial taxa and between microbes and hosts under disease challenges.

Acropora valida and *Turbinaria peltata* are two morphologically distinct species of reef-building corals that are widespread in tropical and subtropical seas of the Indo-Pacific Ocean [38]. They are the dominant species of coral reefs-building corals in coral communities of Weizhou Island in Beibu Gulf, South China Sea [39, 40]. While prior research has indicated that massive corals demonstrate greater adaptability or resistance to environmental changes compared to branching corals, it is more compelling to investigate the mechanisms underlying differences in coral disease resistance or tolerance from the standpoint of the coral holobiont. This study utilized *A. valida* and *T. peltata* to conduct research on the stress response to *V. coralliilyticus*. The phenotypic, microbiome and transcriptional response of *A. valida* and *T. peltata* under *V. coralliilyticus* stress were investigated using high-throughput sequencing of 16S rRNA gene, ITS sequencing and RNA-seq to elucidate the mechanisms underlying interspecific differences in disease resistance. Our study provides novel insights into the coral disease resistance and establishes a theoretical foundation for tackling the crisis of global coral reef degradation.

Methods

Coral samples collection

A. valida and *T. peltata* in good growth conditions were collected from coral communities in Weizhou Island (Guangxi, China) in September 2021. Divers utilized hammers to knock out original coral samples from healthy coral communities. The specimens were expeditiously stored in sample bags containing seawater from the sampling site and promptly transported to Guangxi Laboratory on the Study of Coral Reef in the South China Sea. The raw coral specimens were accurately sectioned into pieces of approximately 4 cm in length and width and placed in experimental tanks under similar conditions for a recovery period of 14 days.

V. coralliilyticus challenge experiment

V. coralliilyticus was cultured in MA medium (MacConkey agar medium) at 29℃ and 160 rpm for 24 h. The concentration of *V. coralliilyticus* was determined according to the methodology outlined by Okwadha [41]. *V. coralliilyticus* was collected by centrifugation at 8000 g and resuspended twice in 0.22 μm-filtered sterile seawater to remove residual medium. We used 12 tanks containing 1000 mL of fresh seawater as inoculation pools, each with one coral sample. Experimental and control groups were set up for each coral species, with each group consisting of three biological replicates. The experimental groups (AVE for *A. valida* and TPE for *T. peltata*) were inoculated with *V. coralliilyticus* at a final concentration of 1×10^5 CFU mL^{-1} (Colony forming unit per mL), while the control groups (AVC for *A. valida* and TPC for *T. peltata*) received an equivalent volume of 0.22 μm-filtered sterile seawater. The inoculation procedure in present study followed the method described by Rosado et al. [42]. Corals were removed from the tanks, placed on sterile petri dishes and inoculated with prepared *V. coralliilyticus* liquid or an equal volume of 0.22μm-filtered sterile seawater using a pipette gun according to the groups. This process required repeated infiltration of the corals with the pipette gun, and the pipette tip was changed between corals for each inoculation. The inoculation process was maintained for 10 min, and corals were placed back into the tanks after inoculation. The temperature was set to 29 ± 0.5°C, and the water quality conditions were consistent throughout the entire experimental period, with salinity maintained at 34‰, KH at 7.2, pH at 8.1, Ca^{2+} at around 420 ppm, Mg^{2+} at around 1290 ppm, $PO_4{}^{3-}$ at less than 0.03 ppm, $NH_3{}^{+}$ at less than 0.15 ppm, and NO^{3-} at approximately 0 ppm. Salinity was measured using a salinometer (Ousu, Hebei, China), and the remaining indicators were measured using the water test kit (Salifert, Netherlands) in accordance with their instructions. Maximum quantum yield (Fv/Fm) of corals were measured using pulse-amplitude-modulated (PAM) (Walz GmbH, Effeltrich, Germany) according to the method of described by Rosado et al. [42]. This procedure was performed after dark adaption about 40 min each day. 20% of the tank seawater was replaced daily with fresh seawater. Phenotypic responses were systematically recorded post-inoculation, and

samples were collected for data analysis upon the manifestation of notable polyp retraction and tissue lesions in corals. Coral tissues underwent thorough rinsing and collection with 0.22 μm-filtered sterile seawater. The enumeration of Symbiodiniaceae was conducted under a microscope. The coral surface area was determined using the aluminum foil method [43], thereby enabling the calculation of Symbiodiniaceae density. Subsequently, the coral specimens were meticulously preserved in liquid nitrogen for the extraction of DNA and RNA.

DNA extraction, amplification and sequencing of 16S rRNA gene and ITS

Total DNA was extracted using Magnetic Soil and Stool DNA Kit (Tiangen Biotech Co., Ltd., Beijing, China). PCR amplification of the V3-V4 variable region of bacterial 16S rRNA was performed using primer 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') [44]. The ITS region of the fungus was amplified using primers ITS2F (5'-GCATCGATGAAGAACGCAGC-3') and ITS2R (5'-TCCTCCGCTTATTGATATGC-3') [45]. The 16S rRNA amplification procedure involved denaturation at 95°C for 5 min, annealing at 55°C for 30 s, extension at 72°C for 40 s, final extension at 72°C for 7 min in a cyclic fashion over 25 cycles, followed by storage at 4°C. Following amplification, purification of the product was performed using the Monarch DNA Gel Extraction Kit (Hongyue Innovation Technology Co., Ltd, Beijing, China) and quantification with Qsep-400.

Illumina NovaSeq platform (novaseq6000, Illumina, San Diego, USA) was employed for sequencing the purified amplicons at the BioMarker Technology Co. Ltd. (Beijing, China). Raw reads were filtered using Trimmonatic (Version 1.2.11) [46], and primer sequences were identified and removed using Cutadapt (Version 1.9.1) [47] to obtain high-quality reads. FLASH (Version 1.2.7) [48] was employed to merge them, yielding clean reads devoid of primer sequences. Finally, UCHIME (Version 4.2) [49] was utilized to identify and remove chimeric sequences to obtain final effective reads.

Diversity analysis of bacteria, fungi and Symbiodiniaceae

Sequences were clustered using USEARCH (Version 10.0) [50] with a similarity threshold set at 97%, and filtered at a threshold of 0.005% to derive operational taxonomic units (OTUs) [51]. Silva (Release128, http://www.arb-silva.de) [52] and Unite (Release7.2, http://unite.ut.ee/index.php) [53] were utilized for aligning and annotating bacterial and fungal sequences, respectively. QIIME2 (Version 2020.6) [54] was employed for conducting α-diversity analysis of microbial communities. The Chao 1 index quantified bacterial and fungal communities richness, while the Shannon index measured community diversity, with higher values indicating greater community richness and diversity, respectively. QIIME was utilized for the β-diversity of bacterial and fungal communities. Principal co-ordinates analysis (PCoA, based on Bray–Curtis) was utilized to visualize the microbial communities β-diversity, while permutational multivariate analysis of variance (PERMANOVA) was employed to test the significance of differences between groups. Line discriminant analysis (LDA) Effect Size (LEfSe) [55] was applied to identify species with significant intergroup differences. The difference threshold was set at LDA = 3.5, and the larger the LDA indicated that the taxon contributed more significantly to the intergroup differences. We screened for OTUs present in over 80% of the samples as core bacterial microbiome to determine changes in the coral microbial community.

DADA2 was utilized to obtain the amplicon sequence variants (ASVs) from reads processed by QIIME2 (Version 2020.6) [54]. The filtering threshold was set at 0.005%. Subsequently, ASVs sequences were aligned with the ITS2 database using BLASTN [56, 57] to determine the types and composition of the Symbiodiniaceae subclades.

RNA-seq analyses

Total RNA of coral samples was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA). Quantification and quality control of the extracted RNA were conducted using the Nanodrop 2000 (Thermo Scientific) and agent 2100, LabChip GX (Agilent Technologies). Subsequently, the qualified RNA was used to prepare the transcriptome library. The adapter sequences for the primer were as follows: adapter3 = AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC; adapter5 = AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT. The quality of the obtained library was assessed using Agilent Bioanalyzer 2100. The library was sequenced using Illumina Novasaq 6000 to obtain paired-end reads.

Trinity [58] was employed to assemble the clean data to generate the unigene library of this species. DIAMOND [59] was utilized to compare unigene sequences with six databases, including non-redundant protein sequence database (NR), Swiss-Prot, clusterof orthologous group (COG), clusters of orthologous groups from 66 complete genomes (KOG), evolutionary genealogy of genes: non-supervised orthologous groups (eggNOG4.5), kyoto encyclopedia of genes and genomes (KEGG). Gene expression analysis was conducted using RSEM [60], and FPKM [61] was utilized to represent the expression abundance of the corresponding unigene. DESeq2 [62] was employed for identifying differentially expressed genes (DEGs) with a fold change ≥ 2 and a false discovery

rate (FDR) < 0.01). Using "experimental group vs control group" to name differentially expressed gene sets such as AVE vs AVC. The DEGs was subjected to GO enrichment analysis using the topGO R package based on the Kolmogorov-Smirnova test method, with a focus on the biological process.

Statistical analysis

The data in this study are expressed as mean ± standard deviation. The significance of the difference between two groups of data was tested using Student's t-test and Wilcoxon rank-sum test. The significance of the difference between the data between multiple groups was tested using analysis of variance (ANOVA) for parametric and Kruskal–Wallis rank-sum test for non-parametric testing. $P < 0.05$ indicates significant difference between data.

Results

Phenotypic response of corals to the *V. coralliilyticus* stress

A. valida exhibited significant lesion symptoms approximately 7 h after *Vibrio* inoculation, with sever polyp retraction, bleaching and tissue loss. In contrast, *T. peltata* did not exhibit lesions throughout the experiment, with normal polyp activity and no tissue lesions or bleaching (Fig. 1A). Additionally, the Fv/Fm results indicated a significant reduction in the photosynthetic efficiency of *A. valida* following *Vibrio* inoculation (Student's t-test, $p = 0.002$), while the photosynthesis of *T. peltata* was not significantly affected (Student's t-test, $p = 0.882$), consistent with the observed phenotypic response (Table S1). The differential response suggests that *T. peltata* may exhibit greater disease resistance compared to *A. valida*.

Response of bacteria in corals to the *V. coralliilyticus* stress

A total of 1902 OTUs were obtained after clustering, 1618 OTUs for *A. valida* and 1852 OTUs for *T.* peltata (Table S2). α-diversity analyses indicated that *A. valida* exhibited higher bacterial α-diversity compared to *T. peltata* (AVC: TPC, Student's t-test, $p = 0.022$; AVE: TPE, Student's t-test, $p = 0.001$) (Fig. 1B and C; Table S2), highlighting the interspecific differences in the α-diversity of the bacterial communities between the coral species. Throughout the *Vibrio* stress, neither the Chao 1 index nor Shannon index of *A. valida* changed significantly, indicating that bacterial richness and diversity were not significantly affected (Chao1, Student's t-test, $p = 0.463$; Shannon, Student's t-test, $p = 0.590$). Simultaneously, there was a non-significant decrease in the Chao1 index of *T. peltata* (Student's t-test, $p = 0.513$), while the Shannon index exhibited a significant reduction, indicating a significant decrease in bacterial diversity in *T. peltata* (Student's t-test, $p = 0.033$). These results suggest that *Vibrio* inoculation exerted a more substantial effect on the α-diversity of the bacterial community in *T. peltata*.

PCoA revealed statistically significant differences in microbial β-diversity among the four groups (PERMANOVA, $R^2 = 0.759$, $p = 0.001$) (Fig. 1D). *Vibrio* stress did not have a significant impact on the microbial community structure of *A. valida* (PERMANOVA, $R^2 = 0.283$, $p = 0.2$), but it resulted in greater similarity in community structure across samples within the AVE compared to AVC (Fig. S1A). For *T. peltata*, PCoA demonstrated that *Vibrio* inoculation significantly affected the bacterial community, leading to notable differences between TPC and TPE (PERMANOVA, $R^2 = 0.274$, $p = 0.001$) (Fig. S1B). These results suggest that the bacterial community of *T. peltata* exhibits a more significant and flexible response to *Vibrio* stress compared to *A. valida*.

A total of 30 phyla and 332 families of bacteria were identified in the 12 samples (Table S3). At the phylum level, the predominant bacterial phyla Firmicutes and Proteobacteria exhibited the highest relative abundance (RA) in *A. valida* and *T. peltata*, respectively (Fig. 1E; Table S4). *Vibrio* inoculation resulted in a significant increase in the RA of Bacteroidetes, Actinobacteria, and Verrucomicrobia in *A. valida* (Student's t-test, $p = 0.028$, 0.03, and 0.001, respectively), while the RA of Firmicutes, Proteobacteria, and Cyanobacteria decreased (Student's t-test, $p = 0.113$, 0.143, and 0.944, respectively) (Fig. 1E; Table S4). However, *Vibrio* inoculation resulted in an increase in Proteobacteria, Firmicutes, Acidobacteria, Epsilonbacteraeota, and Verrucomicrobia in *T. peltata* (Student's t-test, $p = 0.076$, 0.726, 0.786, 0.160, and 0.538, respectively), while a significant decrease in Bacteroidetes (Student's t-test, $p = 0.004$) (Fig. 1E; Table S4).

Differences in the composition of the dominant bacterial taxa of the two coral species were more pronounced at the family level compare to phylum level. The predominant bacterial families in *A. valida* mainly included Lachnospiraceae, Lactobacillaceae, Ruminococcaceae, and Muribaculaceae, with Lachnospiraceae having the highest RA, *Vibrio* inoculation resulted a significantly increase in the RA of Akkermansiaceae and Muribaculaceae (Student's t-test, $p = 0.003$ and $p = 0.008$, respectively) (Fig. 1F; Table S5). The predominant bacterial families in *T. peltata* include Rhodobacteraceae, Enterobacteriaceae, Vibrionaceae and Flavobacteriaceae, with Rhodobacteraceae exhibiting the highest RA (Fig. 1F; Table S5). *Vibrio* inoculation resulted in a significant decrease in the RA of Flavobacteriaceae (Student's t-test, $p = 0.021$) and an increase in Vibrionaceae, Rhodobacteraceae, Pseudoalteromonadaceae, and *Ruegeria* (Student's t-test, $p = 0.349$, 0.159, 0.298, and 0.464, respectively) (Fig. 2A, B, C and D; Table S5).

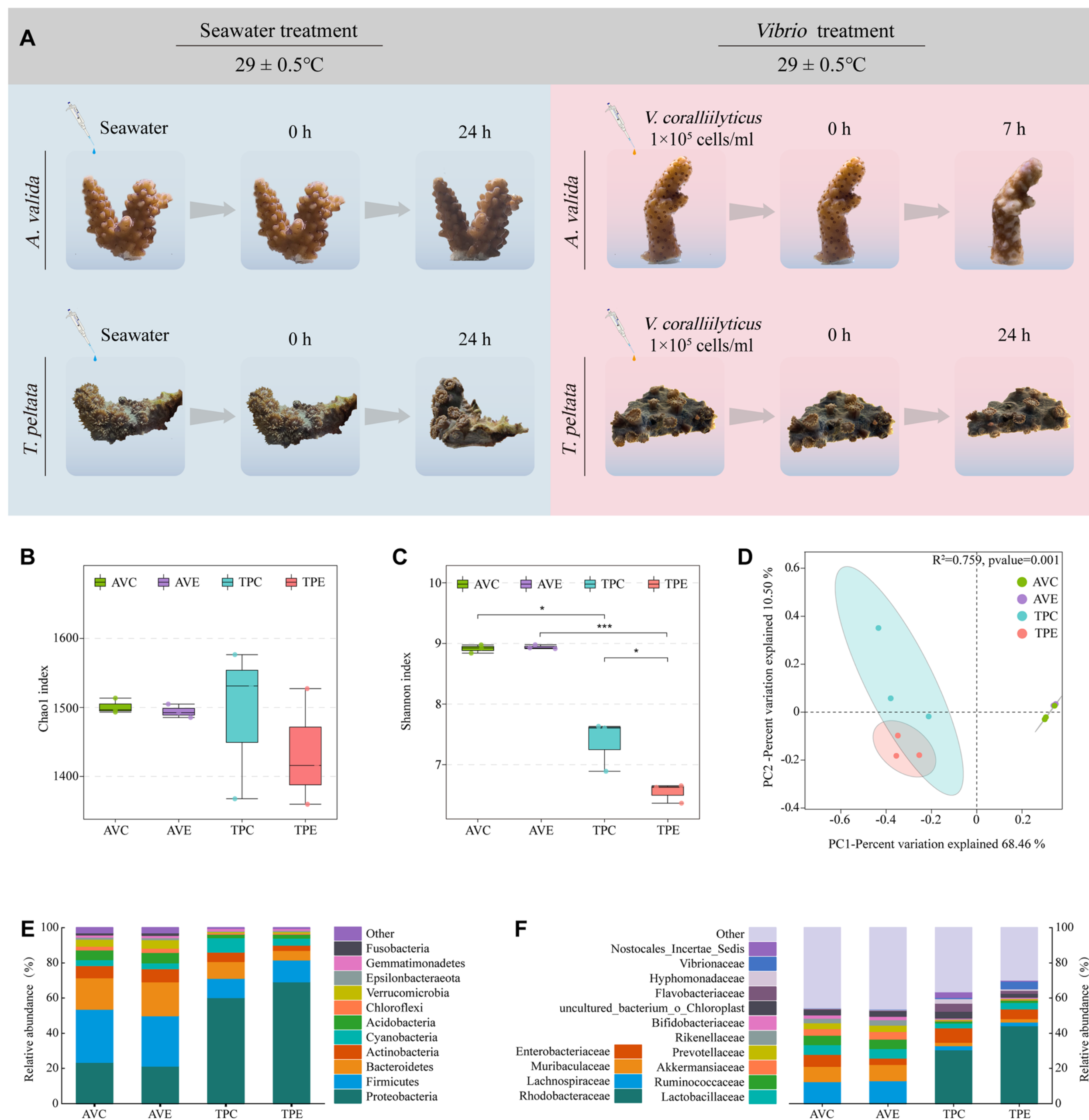

Fig. 1 Phenotypic variation, α-diversity and β-diversity of symbiotic bacterial communities and composition of dominant bacterial taxa in corals. **A** Phenotypic variation charts of the experimental group and the control group of corals. **B** Chao1 index box plots of symbiotic bacterial communities in four groups of corals. **C** Shannon index box plots of symbiotic bacterial communities in four groups of corals. **D** PCoA plots of symbiotic bacterial communities in four groups of corals. **E** The composition and RA changes of dominant bacterial taxa in four groups of corals at the phylum level. **F** The composition and RA changes of dominant bacterial taxa in four groups of corals at the family level

The RA of Lachnospiraceae in *A. valida* was significantly higher than that in *T. peltata* (AVC: TPC, Student's t-test, $p = 0.001$; AVE: TPE, Student's t-test, $p = 1.20E-5$), whereas Rhodobacteraceae, Flavobacteriaceae, and Vibrionaceae exhibited significantly lower levels than that in *T. peltata* (AVC: TPC, Student's t-test, $p = 0.003$, 0.006, and 0.063, respectively; AVE: TPE, Student's t-test, $p = 0.002$, 5.90E-5, and 0.250, respectively) (Table S6). Additionally, at the genus level, the RA of *Ruegeria* was significantly higher in *T. peltata* compared to *A. valida* (AVC: TPC, Student's t-test, $p = 0.027$; AVE: TPE, Student's t-test, $p = 0.033$)

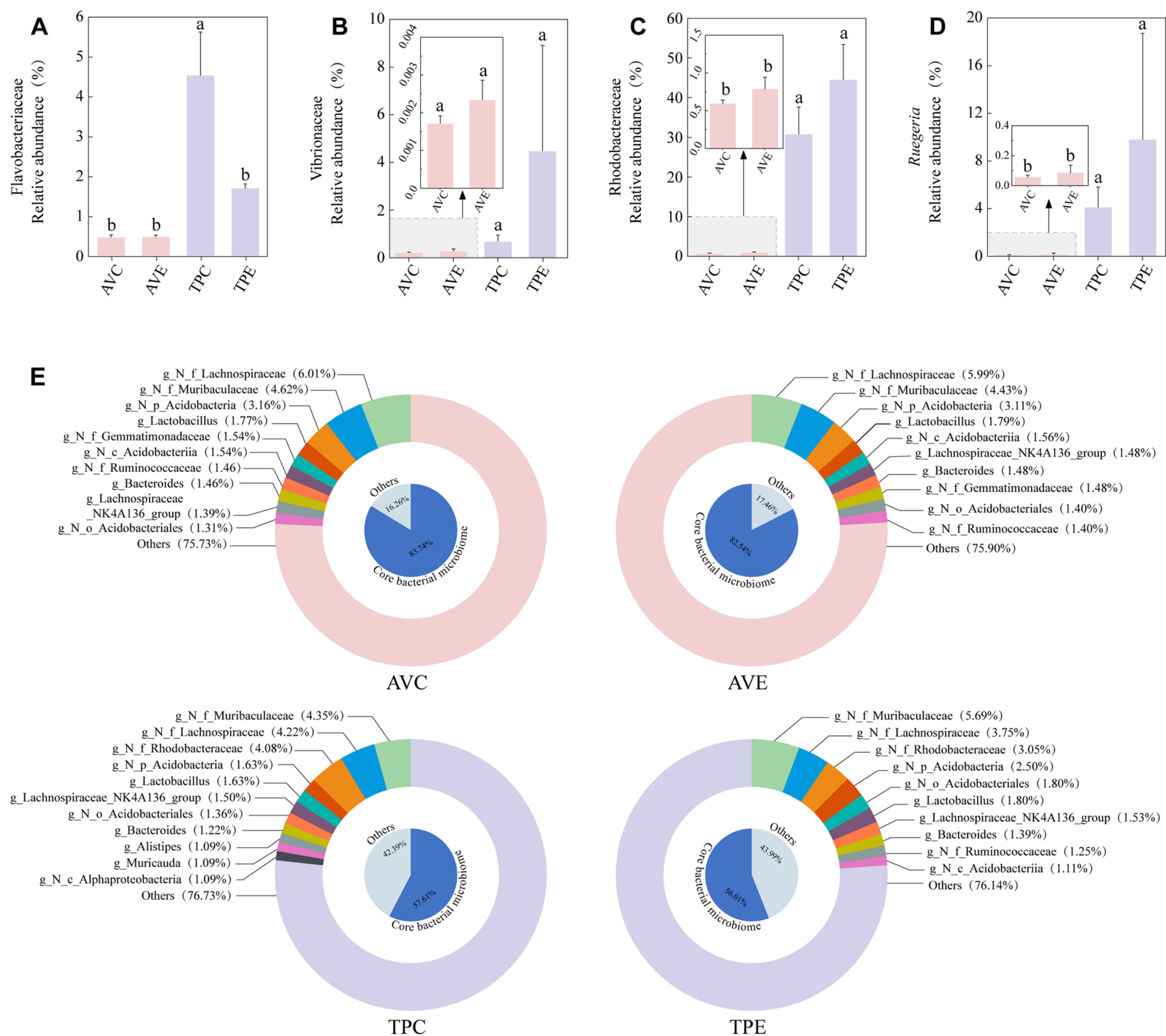

Fig. 2 RA of associated bacterial taxa and composition of core bacterial microbiome in four groups of corals. **A** RA variation of Flavobacteriaceae in four groups of corals. **B** RA variation of Vibronaceae in four groups of corals. **C** RA variation of Rhodobacteraceae in four groups of corals. **D** RA variation of *Ruegeria* in four groups of corals. **E** Statistical charts of RA for core bacterial microbiome in four groups of corals

(Fig. 2D). The high RA of Proteobacteria-related members (e.g., Rhodobacteraceae and Flavobacteriaceae) in *T. peltata* constitutes a notable feature of the bacterial community of this species (Fig. 1E and F). Notably, while the dominant bacterial phyla composition was consistent in both coral species, Proteobacteria were significantly more dominant in *T. peltata* than in *A. valida*, and thus the dominance of the dominant bacterial taxa appeared to be more homogeneous in *A. valida* compared to *T. peltata* (Fig. 1E; Table S7). This pattern was particularly pronounced at the family level (Fig. 1F; Table S6), where family Rhodobacteraceae within the phylum Proteobacteria significantly contributed to the homogeneity of *T. peltata* communities. Furthermore, the homogeneity of the community structure was further exacerbated by the significant increase in RA of taxa such as Rhodobacteraceae as well as Vibrionaceae under *Vibrio* stress.

The proportion of the core bacterial microbiome in AVC and AVE was 83.74% and 82.54%, respectively, which were significantly higher than 57.61% and 56.01% in TPC and TPE, respectively (Fig. 2E). It is worth mentioning that *Vibrio* inoculation resulted in more significant changes in the composition of core bacteria in *T. peltata* compared to *A. valida* (Fig. 2E). These results indicate that the bacterial community of *T. peltata*

demonstrates greater flexibility and inclusivity compared to that of *A. valida*.

LEfSe (LDA = 3.5) revealed that the related members of Gammaproteobacteria, Enterobacteriaceae, and Bacilli were significantly enriched in AVC, while the related members of Bacteroidia, Akkermansiaceae, Actinobacteria, Verrucomicrobia, and Muribaculaceae were significantly enriched in AVE (Fig. 3; Table S8). In *T. peltata*, LEfSe revealed that the related members of Bacteroidetes (Bacteroidia), Flavobacteriaceae, Oscillatoria-coralinae, and Nostocales-Incertae were significantly enriched in the TPC, while the related members of Proteobacteria, *Aliterella_CENA595, Massiliprevotella-massiliensis* and Pseudoalteromonadaceae were significantly enriched in the TPE (Fig. 3; Table S9).

Response of fungus in corals to the *V. coralliilyticus* stress

A total of 202 and 213 OTUs were obtained for the *A. valida* and *T. peltata*, respectively (Table S10). The Chao1 index and Shannon index of the fungal communities in *A. valida* and *T. peltata* exhibited no significant change under *Vibrio* stress (AVC; AVE, Chao1, Student's t-test, $p = 0.427$; AVC; AVE, Shannon, Student's t-test, $p = 0.176$; TPC: TPE, Chao1, Student's t-test, $p = 0.182$; TPC: TPE, Shannon, Student's t-test, $p = 0.315$) (Fig. S2A and B; Table S10), suggesting that *A. valida* and *T. peltata* maintained the relative stability of fungal communities richness and diversity under *Vibrio* stress. PCoA revealed significant intergroup differences in the fungal communities of corals (PERMANOVA, $R^2 = 0.84$, $p = 0.001$) (Fig. S2C), *Vibrio* inoculation significantly increase the heterogeneity of the fungal community in *A. valida* but did not affect the fungal communities in *T. peltata* (PERMANOVA, $R^2 = 0.193$, $p = 0.401$) (Fig. S1C and D). A total of 6 phyla and 11 families were identified in *A. valida* and *T. peltata* (Table S11), these taxa exhibited similar RA across the four groups, suggesting that *Vibrio* inoculation did not result in significant changes in the RA of dominant fungi taxa (Figure S3A and B; Table S12 and S13). These results indicated that the diversity and composition of the fungal communities in both *A. valida* and *T. peltata* were not significantly affected by *Vibrio*.

Response of Symbiodiniaceae in corals to the *V. coralliilyticus* stress

Interspecific differences in Symbiodiniaceae density were detected between *A. valida* and *T. peltata*. Symbiodiniaceae density was significantly higher in *A. valida* than in *T. peltata* in the control groups (Student's t-test, $p = 3.5508\text{E-}7$), with $1.01 \pm 0.0014 \times 10^6$ cells $cm^{-2}$and $2.53 \pm 1.59 \times 10^4$ cells cm^{-2}, respectively. Additionally, the variation of Symbiodiniaceae density in *A. valida* and *T. peltata* exhibited interspecific differences under *Vibrio* stress, with a significant decrease in *A. valida* to $3.24 \pm 1.78 \times 10^4$ cells cm^{-2} (Student's t-test, $p = 4.7433\text{E-}7$) and *T. peltata* decreased slightly to $6.47 \pm 0.83 \times 10^3$ cells cm^{-2} (Student's t-test, $p = 0.170$) (Fig. 4A; Table S14).

Interspecific differences were observed in the compositional diversity of Symbiodiniaceae between *A. valida* and *T. peltata*. At the clade level, *A. valida* only contained *Cladocopium*, whereas *T. peltata* contained *Cladocopium*, *Breviolum*, and *Symbiodinium* (Table S15). At the subclade level, C1 exhibited the highest RA in *A. valida* and *T. peltata*, followed by C1p. The subclades C#, Cspc, C44, and C1m.type2 were exclusively observed in *A. valida*, whereas B1, C1a, C1f, C1.v1b, C41, C1.v1a, and

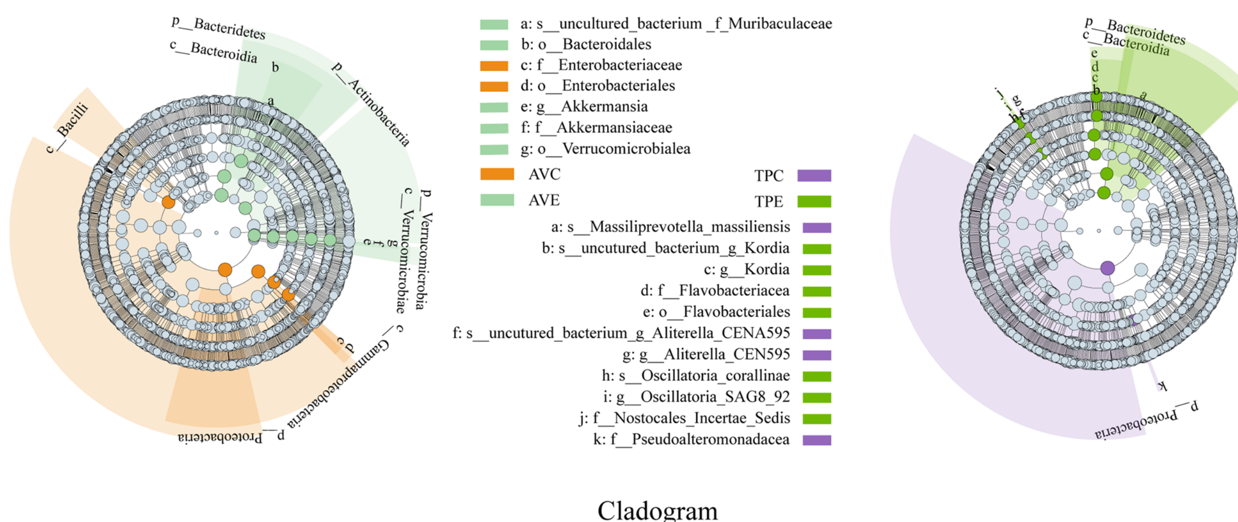

Fig. 3 Cladogram of bacterial taxa significantly enriched in four groups of corals identified by LEfSe

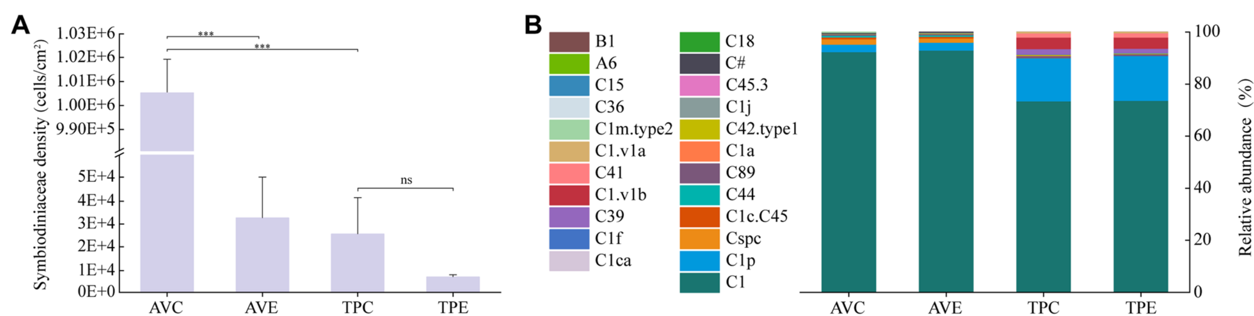

Fig. 4 Variations in symbiotic Symbiodiniaceae density, subclade composition, and RA in four groups of corals. **A** Statistical chart of symbiotic Symbiodiniaceae density in four groups of corals. **B** Composition and RA statistical chart of Symbiodiniaceae subclades in four groups of corals

A6 were present only in *T. peltata* (Fig. 4B; Table S15). Notably, the RA of the C1 in *A. valida* was significantly higher than that in *T. peltata* (AVC: TPC, Student's t-test, $p=2.25E-4$; AVE: TPE, Student's t-test, $p=0.001$), whereas the RA of the C1p in *T. peltata* was significantly higher than that in *A. valida* (AVC: TPC, Student's t-test, $p=2.00E-4$; AVE: TPE, Student's t-test, $p=0.001$) (Fig. S4A).

Vibrio inoculation resulted in a significant decrease in the RA of subclade Cspc (Student's t-test, $p=9.3924E-7$), accompanied by a significant increase in subclades C1, C1j and C42.type1 in *A. valida* (Student's t-test, $p=0.007$, 0.002, and 0.037, respectively). Notably, C1m.type2 disappears following *Vibrio* stress (Figure S4B; Table S15). However, for *T. peltata*, *Vibrio* inoculation did not cause significant changes in the composition and RA of subclades. It is noteworthy that the subclades C1a and A6 disappearing following *Vibrio* stress. Additionally, C18, C1ca, C36, and C15 were exclusively present in the TPE (Figure S4C; Table S15). These results suggest that *Vibrio* stress did not induce a significant reshuffle of the Symbiodiniaceae communities, but it resulted in a decrease in Symbiodiniaceae density.

Transcriptional response of coral hosts to the *V. coralliilyticus* stress

A total of 70,539 unigenes were obtained, constituting approximately 70.5% of the total unigenes (Table S16). A total of 8788 up-regulated genes and 3456 down-regulated genes were identified in *A. valida* (Fig. 5A; Table S17). A total of 763 up-regulated genes and 173 down-regulated genes were identified in *T. peltata* (Fig. 5B; Table S17). GO enrichment analyses revealed the differentially up-regulated genes in *A. valida* were mainly predominantly enriched in the pathways related to energy metabolism and cell differentiation, such as G protein-coupled receptor signaling pathway (GO: 0007186) and hypothalamus cell differentiation (GO: 0021979). Additionally, the differentially up-regulated genes showed a high level of enrichment in immune-related pathway and cell differentiation-related pathways, notably the toll-like receptor signaling pathway (GO: 0002224) and negative regulation of cell differentiation (GO: 0045596) (Fig. 5C; Table 1, Table S18). The differentially down-regulated genes were mainly enriched in the GO terms include DNA integration (GO: 0015074), DNA recombination (GO: 0006310), positive regulation of TOR signaling (GO: 0032008), which are associated with gene expression and immunomodulation. Additionally, certain differentially down-regulated genes were enriched in nitrogen-related cyclic metabolic pathways such as response to amine (GO: 0014075), nitrogen cycle metabolic process (GO: 0071941), urea metabolic process (GO: 0019627) (Fig. 5D; Table 1, Table S18). These results suggest that *A. valida* responds to *Vibrio* stress through the up-regulation of energy metabolism and innate immunity, while *Vibrio* stress inhibits processes such as gene expression and cell regeneration.

The differentially up-regulated genes in *T. peltata* were predominantly enriched in the pathway related to immune regulation and energy metabolism, including negative regulation of receptor signaling pathway via JAK-STAT (GO: 0046426), polysaccharide catabolic process (GO: 0000272), negative regulation of membrane protein ectodomain proteolysis (GO: 0051045), cellular response to potassium ion (GO: 0035865), and D-amino acid metabolic process (GO: 0046416) (Fig. 5E; Table 1, Table S19). The differentially down-regulated genes of *T. peltata* were significantly enriched in the pathway related to ion transport, encompassing regulation of ion transport (GO: 0043269), positive regulation of ion transport (GO: 0043270), anion homeostasis (GO: 0055081), and positive regulation of cellular component organization (GO: 0051130), with the enrichment of regulation of ion transport being the most significant (Fig. 5F; Table 1, Table S19). These results suggest that while *T. peltata* up-regulated the regulation of immune activity, cellular processes such as ion transport are inhibited.

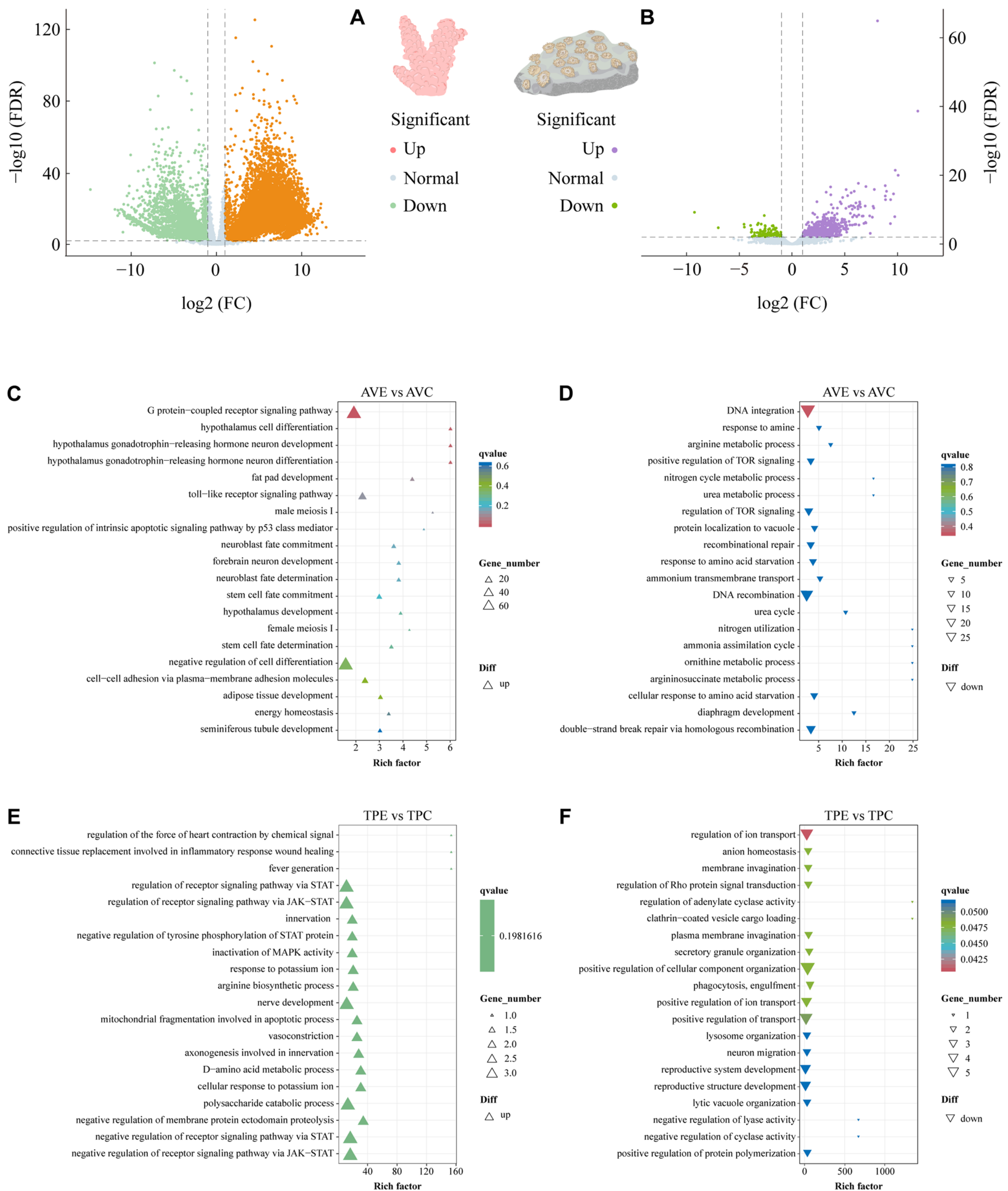

Fig. 5 Differentially expressed genes and GO terms in *A. valida* and *T. peltata*. **A** Volcano plot of differentially expressed genes in *A. valida* after *Vibrio* stress. **B** Volcano plot of differentially expressed genes in *T. peltata* after *Vibrio* stress. **C** Enriched GO terms for differentially up-regulated genes in *A. valida*. **D** Enriched GO terms for differentially down-regulated genes in *A. valida*. **E** Enriched GO terms for differentially up-regulated genes in *T. peltata*. **F** Enriched GO terms for differentially down-regulated genes in *T. peltata*

Table 1 Certain enrichment pathways of DEGs in *A. valida* and *T. peltata*

	Term description	Term ID	Differential regulation	Gene number	q-value
A. valida	G protein-coupled receptor signaling pathway	GO: 0007186	Up	70	0.000448
	Toll-like receptor signaling pathway	GO: 0002224	Up	23	0.123117
	DNA integration	GO: 0015074	Down	27	0.347722
	Positive regulation of TOR signaling	GO: 0032008	Down	7	0.807382
T. peltata	Negative regulation of receptor signaling pathway via JAK-STAT	GO: 0046426	Up	3	0.198126
	D-amino acid metabolic process	GO: 0046416	Up	2	0.198126
	Regulation of ion transport	GO: 0043269	Down	4	0.040816
	Positive regulation of cellular component organization	GO: 0051130	Down	5	0.047706

Discussion

The flexibility of bacterial community and potential beneficial bacteria confer higher disease resistance

Coral symbiotic bacteria play a pivotal role in maintaining host health [63] and are influenced by environmental fluctuations [23]. In this study, *A. valida* harbored higher bacterial α-diversity and exhibited no significant change under *Vibrio* stress, whereas bacteria in *T. peltata* displayed greater flexibility. This is similar to the findings of Tracy et al. [64], who fund that coral bleaching was not necessarily accompanied by changes in α-diversity. Differential variation in α-diversity has been documented in previous studies. It has been shown that bacterial α-diversity decreases under environmental stresses such as tourist activities, high temperatures, and ocean acidification [23, 64–67]. Bacterial communities respond to environmental stresses through dynamics, with bacterial diversity and flexibility serving as a mechanism to buffer the effects of environmental stresses by ensuring complementarity and redundancy of community functions [23]. The reduction in bacterial diversity may represent a strategy employed by communities for mitigating environmental impact. Conversely, infection by pathogen has been recognized as a factor contributing to an increase in bacterial diversity in some studies [20, 24, 25]. Coral symbiotic bacterial communities exhibit characteristics of open systems, wherein the augmentation of bacterial diversity can be ascribed to the host's diminished capacity to regulate the microbial community. Consequently, certain opportunistic bacteria or pathogens may colonize and propagate within the community [5, 63]. Variability of bacterial diversity strongly depends on the underlying community composition of different coral species [68], changes in bacterial diversity may be the result of a combination of coral host, location, and stressors [30]. The differential response of *A. valida* and *T. peltata* to *Vibrio* stress may be closely linked to the inherent composition of the coral symbiotic bacteria. The decrease of bacterial α-diversity in *T. peltata* suggests that its symbiotic bacterial community can respond rapidly to *Vibrio* inoculation, which may represent a process of buffering *Vibrio* stress, whereas bacterial community of *A. valida* responds with a hysteresis, suggesting that the bacterial community in *T. peltata* exhibits a more flexibility compared to that of *A. valida*.

β-diversity analyses revealed that *Vibrio* stress induced a shift in the structure of coral symbiotic bacterial communities, transitioning from a state of higher heterogeneity to relative homogeneity in composition and comparable structural patterns. This is consistent with two recent studies, both of which found increased bacterial community similarity in diseased corals compared to their healthy counterparts [26, 69]. Although certain studies have suggested that environmental stresses may result in heightened heterogeneity and subsequently increased β-diversity in bacterial communities [19, 67], this could signify a down-regulation of the host's regulatory capacity and the disruption of microbial community homeostasis [63]. Changes in the structure of the microbial community may serve as an adaptive response for corals in the face of a fluctuating environment. The observed homogenization of community under the disease stress suggests the prevalence of influential factors, such as pathogens, opportunistic organisms, or coral probiotics [70]. Bacterial communities categorized as healthy or pathogenic exhibit two distinct yet highly competitive community structures. Specific microorganisms effectively resist competitive pressures, resulting in more homogeneous microbial communities [26]. The homogenization of microbial communities may arise from self-regulation. Therefore, *T. peltata* exhibited more pronounced changes in community structure compared to *A. valida*, indicating a more positive response to pathogen stress, which could contribute to swift response and defense against pathogens.

A. valida and *T. peltata* maintained a consistently stable composition and ratios within the core bacterial

microbiome. Approximately 50% of non-core bacterial microbiome (Sporadic symbionts) was present in *T. peltata*. While certain studies have proposed that sporadic symbionts may arise from environmental stochastic event or stress [71], similar proportions of sporadic symbionts were observed in both TPC and TPE. Sporadic symbionts exhibit variability across species, habitat, season, and life stage [71], and they may play a crucial role in maintaining the functional integrity of coral symbiont and adapting to specific environmental stresses [17]. The abundant presence of sporadic symbionts in *T. peltata* implies that its bacterial community is more flexible than that of *A. valida*, demonstrating greater inclusiveness within the bacterial community, which endows the host with a higher bacterial community plasticity and a faster response to *Vibrio* stress.

Analyses of diversity and core bacterial microbiome revealed the host specificity and dynamics of coral symbiotic microbes. *T. peltata* exhibited more pronounced bacterial dynamics in terms of diversity and community structure, enabling it to withstand the *Vibrio* stress. In contrast, *A. valida* showed less fluctuation in bacterial communities but failed to resist *Vibrio*. This finding aligns with the recent research conducted by Pogoreutz et al. [29], which observed coral bleaching and mortality despite the stability of bacterial communities in *Pocillopora verrucosa* exposed to excessive organic nutrients. They suggested that more flexible bacterial communities may help the holobiont to respond or acclimatize to rapid environment changes [29]. This observation was highly similar to findings of Nicholas et al. [72], who found that corals with smaller microbial community dynamics under disease stress exhibited higher susceptibility, while those with more pronounced dynamics in bacterial community demonstrated greater disease resistance. Alterations in coral symbiotic microbial communities may lead to microbial dysbiosis [17]. Nonetheless, the dynamics of microbial communities serve as a crucial mechanism empowering corals to not only withstand environmental stresses but also adapt adeptly to changes in their surroundings [30]. Attributed to the greater dynamism of the bacterial community, *T. peltata* emerges as inherently more flexible in its response to pathogenic challenges, demonstrating a capacity to withstand pronounced fluctuations within the microbial community. Conversely, the relative stability of the bacterial community in *A. valida* may suggest a delayed responsiveness, indicating at a lower threshold for microbial dysbiosis and a rapid die-off in response to pathogens infection.

Alterations in the abundance of associated bacterial taxa reflect the dynamic nature of the microbial community and carry significant implications for host disease resistance. The high abundance of Rhodobacteraceae, Flavobacteriaceae in *T. peltata* suggests that their status as resident members within the bacterial community, which is consistent with previous studies [7, 15, 73]. Related members of Rhodobacteraceae play critical roles in nitrogen fixation and pathogen defense [17, 74]. Related members of the Flavobacteriaceae reduce the production of reactive oxygen species by Symbiodiniaceae to protect the photosynthetic mechanism [75], and they also produce antimicrobial substances like tropodithitic acid through the metabolization of DMSP, thereby inhibiting the proliferation of pathogens [76, 77]. Furthermore, *Ruegeria* has been recognized as a coral probiotic that produces antimicrobial compounds to inhibit pathogenic bacteria, playing an important role in defense against pathogens [77–79], and similar antimicrobial properties were found in related members of Pseudoalteromonadaceae [77, 80]. The significant change the RA of these dominant bacterial taxa in *T. peltata* potentially reflects the competition between potential beneficial bacteria and pathogenic bacteria. The proliferation of these taxa may reduce the competitiveness of the pathogenic *Vibrio* within the community, potentially reshaping the microbial community into a more beneficial state. However, these potential pathogen-resistant bacterial are present in *A. valida* bacterial community with an extremely low abundance and sluggish response, which is not conducive to resist pathogenic infection. Therefore, greater bacterial community flexibility, dysbiosis thresholds, and the abundance of potential beneficial bacteria contribute to *T. peltata* showing better resistance to *V. coralliilyticus*.

Coral disease resistance is influenced by the availability of energy driven by Symbiodiniaceae

Statistical analyses of Symbiodiniaceae densities and photosynthetic efficiencies consistently demonstrated that *Vibrio* inoculation severely disrupted the symbiotic relationship between Symbiodiniaceae and *A. valida.* Symbiodiniaceae transfer approximately 60–80% of the energy to the host through photosynthesis, supporting vital physiological processes such as reproduction, respiration and calcification [17]. Furthermore, numerous studies indicated that the availability of the organic nutrients potentially contributed to shaping coral resistance and resilience, with stable transportation of those nutrients benefiting coral tolerance and resilience [11, 81]. The severe disruption of this symbiotic relationship in *A. valida* undoubtedly interrupts energy transfer and potentially exacerbates host mortality. Conversely, *T. peltata* sustained a relatively constant Symbiodiniaceae density and photosynthetic efficiency under *Vibrio* stress, indicating that the energy transfer driven by Symbiodiniaceae was not significantly affected. Its robust polyps confer

stronger heterotrophic feeding capability, which benefits *T. peltata* by supplementing the energy deficiency caused by the slight loss of Symbiodiniaceae as well as improves tolerance to environmental stress [11, 81]. Furthermore, the thicker tissues of *T. peltata* may store more substantial energy reserves, contributing to coral disease resistance and resilience [82]. It is noteworthy that the popularity of the aluminium foil method for measuring coral surface area in many studies is undeniably less accurate than the 3D modelling method, and therefore there is an unavoidable and objective inaccuracy in the calculation of the Symbiodiniaceae density based on this method [83–85]. The application of the 3D modelling method for surface area measurements in further studies may contribute to revealing more details of the variation in Symbiodiniaceae density.

The composition of Symbiodiniaceae subclades was not significantly alter by *Vibrio* inoculation. Although previous studies have found that some corals were able to shuffle, switch and recombine Symbiodiniaceae subclades to improve host resistance and resilience to withstand environmental stress [57, 66, 84], no recombination of dominant Symbiodiniaceae subclades was observed in present study, suggesting that short term pathogen stress may not exert sufficient influence to alter the Symbiodiniaceae community structure. Additionally, a plausible explanation is coral hosts usually establish symbiotic relationships with a single genetic clone of Symbiodiniaceae [67, 86], it will occupy an advantage once the clonal Symbiodiniaceae lineage is established in the host, which may represent a crucial mechanism to the coral's environmental adaptation over a long-time evolution [87]. However, certain Symbiodiniaceae subclades disappeared after *Vibrio* stress, including C1m.type2, C1a, and A6. Coral host may selectively expel redundant or harmful Symbiodiniaceae to maintain a stable symbiotic relationship in response to environmental pressure [88].

The present study offers a novel perspective on investigating interspecific differences within Symbiodiniaceae communities and their responses to pathogen pressure through the ASV method. Nevertheless, numerous challenges persist in the utilization of ITS2, encompassing intragenomic variation (IGV), pseudogenes, and PCR artifacts, albeit these limitations have been notably mitigated through the utilization of next-generation sequencing and the data processing method for Symbiodiniaceae ITS2 NGS data established by Arif et al. [89]. Consequently, relying solely on the ASV method for assessing Symbiodiniaceae diversity may introduce some uncertainties. In future research, the adoption of Symportal developed by Hume et al. [90] may facilitate a more comprehensive exploration of the nuanced alterations within the Symbiodiniaceae community under disease pressure.

Coral disease resistance is shaped by the host's energy availability and immune activity

The differentially up-regulated genes of *A. valida* showed the most significant enrichment in G protein-coupled receptor signaling pathway. G protein-coupled receptor (GPCR) represent the largest and most diverse membrane receptor in eukaryotic organisms, closely tied to the perception and health of organisms to the external environment [91]. The pathway was activated by *Vibrio* stress to regulate cellular metabolism through downstream pathways such as cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) signaling pathways, involving the degradation of glycogen into glucose for various cell biological processes [91]. The significant up-regulation of the GPCR signaling pathway is likely to correlate with the massive loss of the symbiotic Symbiodiniaceae. The loss of Symbiodiniaceae leads to a reduction in energy availability, with the host released stored energy through accelerating the degradation of glycogen to compensate for the energy shortfall. Notably, the GPCR signaling pathway will further activates apoptosis. Previous studies have identified a significant correlation between the disease-related mortality of susceptible corals and the excessive activation of apoptosis [34]. Therefore, the significant up-regulation of this pathway could exacerbate immune dysregulation and mortality. Furthermore, a considerable number of differentially up-regulated genes were enriched in the toll-like receptor signaling pathway. Toll-like receptors (TLR) serve as pattern recognition receptors and are essential components of the biological innate immune system, identifying exogenous pathogens to initiate an immune response [92]. The up-regulation of this pathway suggest that *Vibrio* stress stimulates the innate immune response in *A. valida*. These findings were consistent with the perspective that heightened immune activity correlates with increased energy demands, the coral holobiont sustains elevated immunity levels by augmenting the energy supply from Symbiodiniaceae [93]. The maintenance of homeostasis in the coral holobiont through immunity may be intricately linked to its energy supply, suggesting that this correlative mechanism could enhance the host's resilience amidst environmental fluctuations. However, the massive loss of symbiotic Symbiodiniaceae renders *A. valida* unable to meet the high energy demands of the immune response. Moreover, TOR is involved not only in regulating cell growth and metabolism but also in influencing coral innate immune activities through the regulation of autophagy [34]. Consequently, the down-regulation of the TOR-related pathways in *A. valida* result in the dysregulation of metabolic processes and immune homeostasis. Furthermore, the inhibition of

pathways related to DNA integration and nitrogen metabolism reduced the disease resistance of *A. valida*.

The differentially up-regulated genes of *T. peltata* were predominantly enriched in GO terms related to immune regulation and metabolism of organic substances. The JAK-STAT pathway plays an essential role in cell proliferation, differentiation, and immunomodulation [94]. Abnormal activation of this pathway disrupts immune regulation in the organism, resulting in immune disorders, inflammation, and the development of cancer [94–96]. The negative regulatory mechanism of JAK-STAT pathway represents a crucial mechanism involved in the regulation of immune homeostasis, which contributes to the prevention of immune disorders of the organism. Numerous studies have demonstrated that the JAK inhibitors effectively suppress the abnormal expression of the JAK-STAT pathway to prevent immune diseases [97, 98]. The excessive immune stress in coral host under disease conditions may trigger apoptosis, which may induce further mortality [34]. Therefore, the

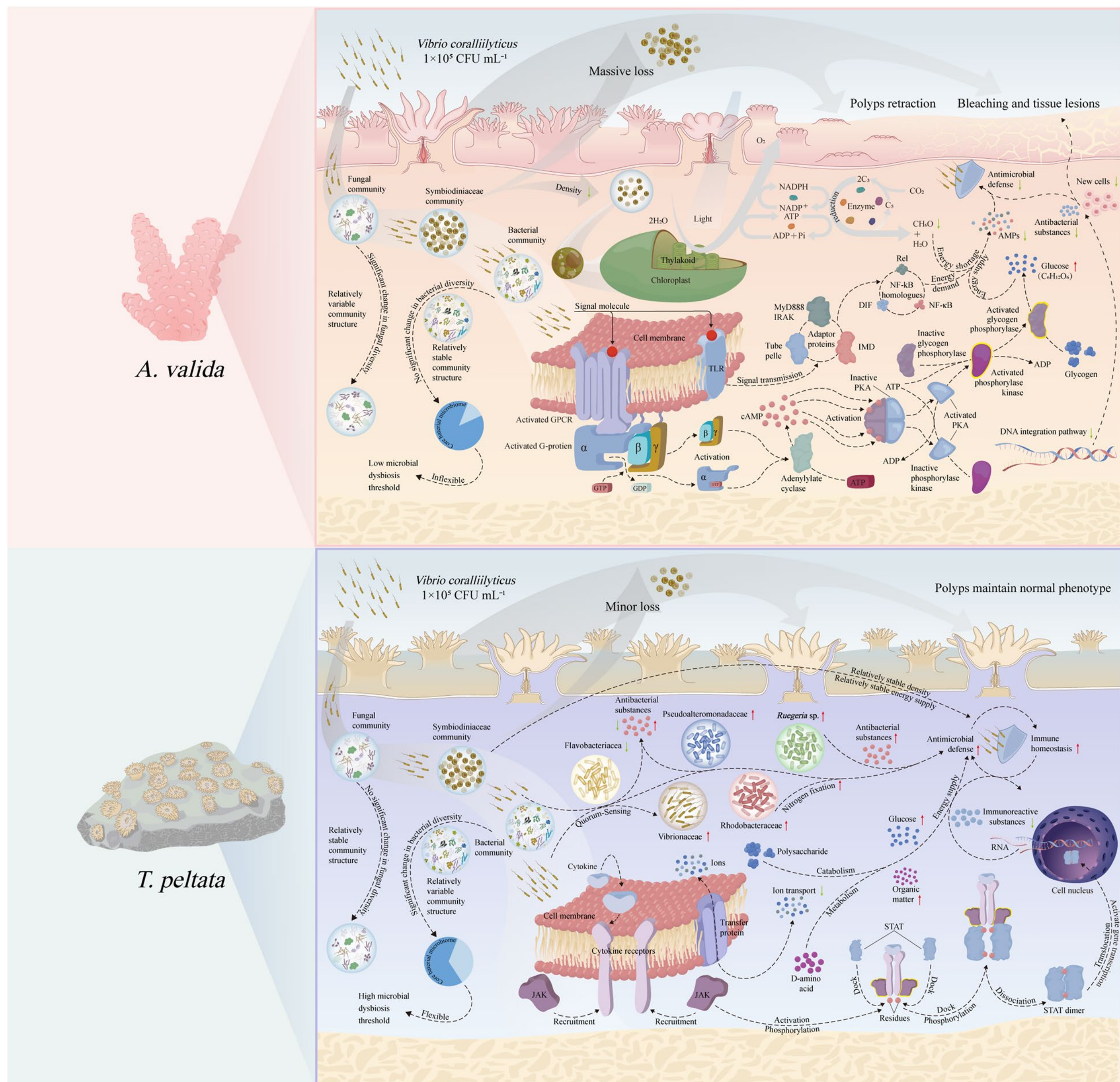

Fig. 6 Response mechanisms in coral holobionts under *V. coralliilyticus* stress. GPCR, G protein-coupled receptor; TLR, Toll-like receptor; cAMP, Cyclic adenosine monophosphate; PKA, protein kinase A; AMPs, antimicrobial peptides; JAK, Janus Kinase; STAT, signal transducer and activator of transcription; DMSP, Dimethylsulfoniopropionate

up-regulation of negative regulation of receptor signaling pathway via JAK-STAT in *T. peltata* under *Vibrio* stress likely contributes to host maintenance of immune homeostasis for disease prevention. Additionally, the up-regulation of pathways related to polysaccharide and protein metabolism suggests that *T. peltata* may enhance energy supply for the coral holobiont by accelerating nutrient metabolism under pathogen stress. This process helps alleviate energy deprivation caused by the minor loss of Symbiodiniaceae, maintaining the stability of carbon and nitrogen cycles in the coral holobiont, thereby enhancing coral disease resistance and resilience. Conversely, pathways related to ion transport were particularly prominent among down-regulated pathways. Cells maintain intracellular ion homeostasis by regulating the transmembrane transport of ions through ion channels and transport carriers [99], which is essential for coral physiological processes such as photosynthesis and calcification. The up-regulation of genes related to ion transport may enhance coral resistance to ocean acidification, entailing a trade-off with the regulation metabolic processes [100]. Certain corals either up-regulate metabolic pathways to acquire sufficient energy for resisting environmental stress (e.g., ocean acidification) or slow down their metabolic activities for awaiting environmental improvement [101]. However, the reduction of metabolic activities is accompanied by the up-regulation of ion transport pathways to minimize energy loss by slowing down other metabolic activities, given the significant energy demands of cellular ion transport during certain environmental stress [100]. This trade-off mechanism was observed in our study, wherein *T. peltata* maintains the expression of vital pathways crucial for coral resistance to pathogens and improves resilience by selectively down-regulating less useful pathways for resisting environmental stresses.

Although the up-regulation of pathways related to polysaccharide metabolism in *A. valida*, it inadequately compensates for the energy deficit caused by the substantial loss of Symbiodiniaceae, which is detrimental to the host's innate immune activity and defense against pathogens. Furthermore, the simultaneous suppression of cellular regeneration and synthesis of antimicrobial substances, along with the dysregulation of metabolic and immune processes, hinders the organism's ability to resist pathogens. While *T. peltata* exhibited a greater ability to regulate the immune system, averting immune dysregulation induced by *Vibrio* stress, and it obtained energy replenishment by up-regulating specific organic metabolic pathways, which involve a trade-off with regulation of certain pathways such as ion transport.

Conclusions

In summary, the present study highlights that the heightened dynamics and flexibility of the symbiotic bacterial community and the antimicrobial activity of coral potential beneficial bacteria contribute to a swift response and defense against pathogens. The substantial depletion of symbiotic Symbiodiniaceae leads to an insufficient energy supply, which poses a challenge for the host to compensate for by accelerating nutrient metabolism and is detrimental to innate immune activity. Maintaining the homeostasis in both symbiotic Symbiodiniaceae density and immune activity bestows upon corals enhanced disease resistance and resilience (Fig. 6). Our findings provide valuable insights into the mechanisms linking alterations in the coral microbiome, transcriptional regulation and disease resistance amid disease stress, and provide a molecular theoretical foundation for the study of coral disease resistance.

Abbreviations

DMSP	Dimethylsulfoniopropionate
MA medium	MacConkey agar medium
CFU	Colony forming unit
AVC	Control group of *A. valida*
AVE	Experimental group of *A. valida*
TPC	Control group of *T. peltata*
TPE	Experimental group of *T. peltata*
Fv/Fm	Maximum quantum yield
OTU	Operational taxonomic unit
RA	Relative abundance
PCoA	Principal co-ordinates analysis
PERMANOVA	Permutational multivariate analysis of variance
LDA	Line discriminant analysis
LEfSe	Line discriminant analysis Effect Size
ASV	Amplicon sequence variant
NR	Non-redundant protein sequence database
COG	Clusterof orthologous group
KOG	Clusters of orthologous groups from 66 complete genomes
eggNOG4.5	Evolutionary genealogy of genesnon-supervised orthologous groups
KEGG	Kyoto encyclopedia of genes and genomes
DEG	Differentially expressed gene
FDR	False discovery rate
ANOVA	Analysis of variance
GPCR	G protein-coupled receptor
cAMP	Cyclic adenosine monophosphate
PKA	Protein kinase A
TLR	Toll-like receptor
AMPs	Antimicrobial peptides
JAK	Janus Kinase
STAT	Signal transducer and activator of transcription

Supplementary Information

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Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

Supplementary Material 4.

Supplementary Material 5.

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Authors' contributions
We have taken appropriate measures to ensure the accuracy and reliability of the data and have followed the principles of scientific integrity in data analysis and result presentation. H.S. conceived the research; C.L., J.Z. and Q.C. contributed the materials; X.H. and C.L. performed all experiments; Y.L., L.Z. and X.Q. identified coral species; X.H. and H.S. wrote the manuscript; all authors edited and approved the manuscript. The authors declare that they have no conflict of interest.

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Availability of data and materials
The datasets generated during the current study have been deposited and are publicly available in the Sequence Read Archive repository under BioProject ID PRJNA1044715, PRJNA1044737, and PRJNA1044533.

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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