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Alleviating coral thermal stress via inoculation with quorum quenching bacteria

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Abstract

In the background of global warming, coral bleaching induced by elevated seawater temperature is the primary cause of coral reef degradation. Coral microbiome engineering using the beneficial microorganisms for corals (BMCs) has become a hot spot in the field of coral reef conservation and restoration. Investigating the potential of alleviating thermal stress by quorum quenching (QQ) bacteria may provide more tools for coral microbial engineering remediation. In this study, QQ bacteria strain *Pseudoalteromonas piscicida* SCSIO 43740 was screened among 75 coral-derived bacterial strains, and its quorum sensing inhibitor (QSI) compound was isolated and identified as 2,4-di-tert-butylphenol (2,4-DTBP). Then, the thermal stress alleviating potential of QQ bacteria on coral *Pocillopora damicornis* was tested by a 30-day controlled experiment with three different treatments: control group (Con: 29°C), high temperature group (HT: 31°C), and the group of high temperature with QQ bacteria inoculation (HTQQ: 31°C + QQ bacteria). The results showed that QQ bacteria SCSIO 43740 inoculation can significantly mitigate the loss of symbiotic algae and impairment of photosynthesis efficiency of coral *P. damicornis* under thermal stress. In addition, QQ bacteria inoculation suppressed the coral microbial community beta-dispersion and improved the stability of microbial co-occurrence network under thermal stress. It was suggested that QQ bacteria inoculation can alleviate coral thermal stress via reshaping microbial interaction and maintain community stability of coral microbiome. This study provided new evidence for the probiotic function of QQ bacteria in corals, which shedding light on the development of new microbiological tools for coral reef conservation.

Introduction

Coral reef ecosystems, which are called “the tropical rainforests of the ocean”, are among the most productive and biodiverse ecosystems on earth (Chen et al. 2015). A variety of symbiotic relationships maintain a high total primary productivity in coral reef ecosystems (Wang et al. 2022). Despite covering only 0.1% of the seafloor, coral reefs provide habitats for about 25% of the marine organism species (Anthony 2016; Hill et al. 2021). Coral reef ecosystems provide numerous ecological and social services, including renewable resources such as fisheries coastline protection, tourism, and natural medicines (Carlson et al. 2021; Haas et al. 2015; Petersen et al. 2020). Today, more than one hundred countries have coral reefs along their coastlines, and 450 million people live near coral reefs (Morrison et al. 2019). However, under the impact of both global and regional pressures, coral reef ecosystems around the world have degraded dramatically since 1970s of the last century (ICRI 2021). In the background of global warming, coral bleaching induced by elevated seawater temperature is one of the major causes of coral reef degradation (Eakin et al. 2019).

The thermal sensitivity of coral-algal symbiosis is well documented. Coral bleaching occurs when corals are exposed to external stresses, such as high temperature, resulting in the loss of symbiont algae or photosynthetic pigments (Bourne et al. 2016). The loss of symbiont algae reduces the transfer of carbohydrates and oxygen to the coral host, which are produced by photosynthesis of symbiont algae (Grottoli et al. 2006; Rosenberg et al. 2007). As the supply of material and energy decreases, corals

become fragile and susceptible to mortality. High temperature will affect the balance of the symbiotic relationship between coral and symbiont algae. Under heat stress, the reaction center D1 protein was degraded and ribulose-1,5-bisphosphate carboxylase activity decreased, causing damage to photosystem II (PSII) activity, and blocking the Calvin cycle in the symbiont algae (Lesser 2006). Damage to the thylakoid membrane leads to an increase in the electron transport rate at the PSII receptor side and a decrease in the photochemical maximum quantum yield of the reaction center (Nesa and Hidaka 2009; Stambler 2011). At low oxygen concentrations, oxygen free radicals decrease and reactive oxygen species (ROS) increase, making coral host cells damaged and susceptible to pathogen infection (Banin et al. 2003; Lesser 2006).

Under thermal stress, coral health is affected not only by bleaching related nutrient and energy depletion, but also by the instability of coral associated microbial communities, which exacerbate coral bleaching. Previous studies have shown that corals produce more DMSP under thermal stress (Garren et al. 2014; Raina et al. 2013). As a chemokine, DMSP attracts more invasion of pathogens such as *V. corallilyticus*, making corals more vulnerable under thermal stress (Garren et al. 2014). Tait et al. found that temperature had a significant influence on the production of short-chain *N*-acyl homoserine lactone (AHL) and pathogen virulence by quorum sensing (QS) bacterial strains (Tait et al. 2010). The production of AHLs by QS bacteria in corals at 30°C was significantly higher than that at 24°C, which may enhance the infection of Vibrio associated black belt disease (Bhedi et al. 2017). Studies have shown that pathogenic bacterial infection can be mitigated by quorum quenching (QQ) bacteria and quorum sensing inhibitor (QSI) compounds at normal temperature (Torres et al. 2016; Zhou et al. 2020). Therefore, regulating the microbial QS process may help to reduce the influence of pathogenic bacteria and alleviate coral bleaching under thermal stress.

Coral microbiome engineering using coral beneficial microorganisms (BMCs) is a promising approach to improve coral health (Li et al. 2023). Inoculation of corals with BMCs has become a hot topic in the field of coral reef ecological restoration (Peixoto et al. 2017; Peixoto et al. 2021; Rosado et al. 2019). Many case studies have proved the effectiveness of coral microbiome engineering by inoculating BMCs with various strategies, such as enhancing nutrient supply (Zhang et al. 2021a)(Zhang et al. 2021a), improving coral tolerance to pollutants (Silva et al. 2021), participating in coral development (Petersen et al. 2021; Sharp et al. 2015; Zhang et al. 2021b), controlling pathogens and diseases (Welsh et al. 2017; Zhou et al. 2020), and alleviating heat-induced bleaching (Rosado et al. 2019; Santoro et al. 2021). Previous studies have shown that QQ bacteria have probiotic effects on corals at normal temperatures (Torres et al. 2016; Zhou et al. 2020). However, whether the microbial engineering strategy of interfering with the QS process by inoculating QQ bacteria can alleviate thermal stress in corals remains unknown. Verifying the potential of QQ bacteria to alleviate thermal stress in corals may provide more tools for microbial remediation of coral reefs.

In this study, we aim to (1) know whether QQ bacteria can relieve coral physiological impairment under thermal stress and (2) understand how QQ bacteria affect coral associated microbial communities under thermal stress. To fill this knowledge gap, we screened a QSI-producing bacterial strain from coral, then

inoculated it into corals under elevated temperatures, and tracked the responses of host physiological parameters and microbial communities to evaluate the probiotic ability of QQ bacteria in alleviating coral thermal stress.

Materials and methods

QQ bacteria isolation and screen

Coral samples for bacteria isolation were collected from the Luhuitou fringing reef in Sanya, China (109°28'27" E, 18°12'19" N) on December, 2019. Coral fragments of *Pocillopora damicornis*, *Acropora millepora*, *Galaxea fascicularis*, *Acopora formosa* and *Acropora digitifera* were collected, flushed with sterilized seawater and homogenized by grinding and stirring. The homogenates were then serially diluted (10^{-1} to 10^{-9} times) in sterilized seawater, and 100 μL of the dilutions were spread onto marine agar 2216E and incubated at 28°C for 2–7 days. Single bacterial colonies were picked and streaked onto new plates to obtain pure strains.

To screen the obtained strains with QQ activity, a disc diffusion assay (Bauer et al. 1966) was performed using the biosensor strain *Chromobacterium violaceum* CV026 (McLean et al. 2004). Briefly, *C. violaceum* CV026 was cultured in Luria-Bertani broth (LB: consisting of 10 g L^{-1} tryptone, 5 g L^{-1} yeast extract, 10 g L^{-1} NaCl, sterilized at 121°C) supplemented with 25 μg·mL^{-1} kanamycin. *N*-hexanoyl-L-homoserine lactone (C6-HSL, Sigma-Aldrich, USA) and 2,5-dimethyl-4-hydroxy-3[2H] furanone (Aladdin, China) were prepared by dissolution in methanol. Each 6 mL of overnight reporter strain culture and 0.5 μM C6-HSL were added to 54 mL of LB media containing 0.8% agar until the temperature of the media was about 45°C. Sterile filter paper circles (6 mm diameter) were then placed on the solidified plates for sample loading. Bacterial suspension (10 μL) in 2216E broth was pipetted onto the filter paper for testing. In addition, 10 μL of furanone (0.1 M), methanol solvent and 2216E broth were used as positive, negative and blank controls in this plate-based bioassay. After incubation for 24 h at 28°C, the inhibition of pigment production around the disc (a colorless ring) was checked. Visible colorless haloes around the filter paper circles represent potential positive QQ activity. To ensure reliability, QQ activity screening experiments were repeated three times independently.

Genomic DNA of potential QQ bacterial strain was extracted using the Bacterial DNA Kit (Omega Bio-tek, USA) following the manufacturer's instructions. The 16S rRNA gene was amplified by PCR using the primers 27F (5'AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Weisburg et al. 1991), and sent for sequencing (Tianyi Huiyuan Biotechnology, China). The 16S rRNA gene of the QQ bacterial strain and reference sequences were aligned using Clustal W (Larkin et al. 2007). The phylogenetic tree was constructed by the neighbor-joining method using MEGA software (Tamura et al. 2021).

Extraction and Identification of QSI

The QQ bacterial strain *Pseudoalteromonas* SCSIO 43740 was fermented in10 L of 2216E broth for 72 h (28°C, 180 rpm). The fermentation broth was then extracted twice with an equal volumes of ethyl acetate, by vigorous shaking for 10 minutes. The aqueous fractions were discarded and the organic extract fractions were combined and evaporated in a rotary evaporator at 45°C. The organic residues were dissolved in methanol (Nithya et al. 2010), filtered through 0.22μm filters and concentrated by nitrogen flow. The extracts were then loaded onto a silica gel column chromatography (10g; 100–200 mesh) and eluted with a stepwise system of petroleum ether/ethyl acetate = 1:0, 50:1, 25:1, 9:1, 6:1and 3:1 (v/v, 1 L each). The fractions were grouped according to their thin-layer chromatography (TLC) profile. These single band fractions were evaporated to dryness and tested for QSI activity. After drying, the fractions were dissolved in methanol and tested for QSI activity at 16.4 mg/ml to better discriminate the most active fraction.

As previously described (Cho et al. 2016), nuclear magnetic resonance (NMR, Bruker Biospin, Fällanden, Switzerland) analysis was carried out after reconstitution of the bioactive fraction in deuterated methanol (CD_3OD, Cambridge Isotope Laboratories, Inc), containing tetramethyl silane (TMS) as analytical solvents. NMR spectra were recorded on a Bruker Avance III spectrometer (Bruker Biospin) (500 and 126 MHz for ^{1}H and ^{13}C NMR, respectively) equipped with a 1.7 mm MicroCryoprobe (Bruker Biospin), using the signal of the residual solvent as internal reference (δ_H 3.31 and δ_C 49.15 ppm for CD_3OD). All mass spectra were acquired on a hybrid iontrap time-of-flight mass spectrometer (Bruker Biospin, Fällanden, Switzerland) equipped with an electrospray ionization source (ESI-MS).

Coral culture and experimental design

Ten healthy coral individuals (diameter ~ 25cm) were collected from the Luhuitou fringing reef (Sanya, China). Prior to use, the coral *P. damicornis* was divided into 120 coral fragments (5 cm each) and transferred to a sand-filtered seawater tank. The corals were cultured in the laboratory for 30 days under the same initial environmental conditions (temperature, 29 ± 0.5°C; pH, 8.1 ± 0.05; and salinity, 31.0 ± 0.5‰). All tanks were maintained at 200 μmol/m^2 s^{-1} light in a 12-h light/dark cycle. Figure 1 shows the schematic diagram of this study, which included a control group (Con: 29°C) and a high temperature group (HT: 31°C), and a high temperature with QQ bacteria inoculation group (HTQQ: 31°C + QQ bacteria) (Fig. 1). Coral fragments were randomly assigned to each group.

As previously described (Rosado et al. 2019), after the initial 5-day coral acclimation period at 29°C, in two of the treatments the temperature was gradually increased from day 1 to day 5 until it reached 31°C. The controlled experiment started when the maximum temperature was reached. In each treatment, 5 coral samples were taken from tanks on days 0, 10 and 30. The timing of the addition of experimental strains is shown in Fig. 1. QQ bacteria were added every 10 days, and the final concentration of inoculated QQ bacteria in the tank was 1×10^7 CFU mL^{-1}.

Following live measurements of physiological performance, fragments were snap frozen in liquid nitrogen and stored at -80°C until further processing, when they were airbrushed with 0.22 μm filtered

seawater, homogenized to obtain tissue slurry (Diax 900, Heidolph Instruments) and aliquoted into individual 1 mL samples for measurements of host protein, superoxide dismutase and catalase activity, and symbiont density.

Measurement of coral physiological parameters

A total of five physiological parameters of coral holobionts were assessed at each time point, including photosynthetic efficiency (F_v/F_m), symbiont algal density, total protein content, superoxide dismutase (SOD) activity and catalase (CAT) activity. Symbiont algal density was counted by light microscope (Olympus Biomicroscope, Olympus Japan Ltd). The counts were repeated three times for each sample. Coral photosynthetic efficiency (F_v/F_m) was assessed using the diving-PAM system (Walz GmbH, Effeltrich, Germany), with the glass fiber-optic probe positioned over the oral disc (Ralph et al. 2005; Rosado et al. 2019). To avoid interference from photoinhibition, measurements were taken after 30 min of darkness to ensure full recovery of photosynthetic reaction centers. Coral protein concentration was determined in triplicate per sample using a BCA protein concentration kit (Beyotime Biotechnology, China). SOD concentration was determined in triplicate per sample using a superoxide dismutase activity colorimetric assay kit (Beijing Sollerbauer Technology Co, China). Results are expressed in ‘standard cytochrome c’ SOD units (U), by measuring the ratio of auto-oxidation rates in the presence and absence of the sample (Gardner et al. 2017). CAT concentrations in coral holobionts were determined using the CAT activity assay kit (Beijing Sollerbauer Technology Co, China) following the manufacturer’s instructions. SOD and CAT activities were normalized to protein concentrations.

16S rRNA gene sequencing

The homogenized coral fragment subsamples (~ 1 g) were used for microbial DNA extraction using HiPure Soil DNA Kits (mBio, USA). The quality and quantity of extracted DNA was checked on a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). Prokaryotic 16S rRNA genes were amplified by the primer pair F341 (5'-CCT ACG GGN GGC WGC AG-3') and R806 (5'-GGA CTA CHV GGG TAT CTA AT-3') as described in previous study (Cole et al. 2009). A 6-mer-barcode sequence was linked on the primer for distinguishing different samples. Briefly, the reactions were carried out in a 50 μL volume using a Bio-Rad PCR cycler: pre-denaturation at 94°C for 2 min; 30 cycles of denaturation at 95°C for 10 s, annealing at 62°C for 30 s, and elongation at 68°C for 30 s; with a final extension for 10 min at 68°C. To generate the sequencing libraries, PCR products for each sample were pooled and purified using the AMPure XP Beads (Qiagen, USA) and quantified using Qubit 3.0. The sequencing was performed on a NovaSeq platform (Illumina, USA) using a paired-end 250-bp sequence read run (Gene Denovo, Guangzhou).

Sequencing data were processed using the QIIME 2 pipeline (Bolyen et al. 2019). Sequences were trimmed and filtered according to previously described methods (Callahan et al. 2016). DADA2 is used for quality control, noise reduction and filtering to obtain a preliminary amplicon variation sequence (ASV) table. The original ASV table was rarified to a depth of 7,000 sequences per sample for further

diversity calculation. The ASV sequence taxonomy annotation was conducted by blast against the EzBioCloud 16S database (Yoon et al. 2017).

Microbial network analysis

To evaluate the co-occurrence patterns of coral microbes, network analysis was performed using the Molecular Ecological Network Analyses Pipeline (MENAP) platform based on random matrix theory (http://ieg4.rccc.ou.edu/mena). To reduce network complexity, only the top-class nodes with strong positive ($r > 0.6$) and strong negative ($r < -0.6$) relationships were shown in the network diagrams. The networks were visualized in Cytoscape v3.9.1 (Shannon et al. 2003), with the Network-Analyser tool used to calculate each network's complexity, namely its number of nodes, clustering coefficients, link number, and average connectivity (Berry and Widder 2014). The robustness test is a powerful method to measure network stability (Montesinos-Navarro et al. 2017). We calculated microbial network stability employing natural connectivity (Wu et al. 2019) in R software using the ggClusterNet package.

Statistical analysis

The parameters are presented as mean ± standard error. The data were subjected to a normality test and a variance homogeneity test. One-way analysis of variance (ANOVA) was used to statistically analyze the differences, was marked using the LSD alphabet notation method. The ASV counts were normalized by the metagenomeSeq method (Paulson et al. 2013). Based on the ASV abundance information, principal component analysis (PCoA) was performed to investigate the microbial structure relationship between samples, and the weighted unifrac distance was selected for calculation. The significance of β-diversity difference was determined using ANOSIM tests. The beta-dispersion was represented by the average inner group distance in the weighted unifrac dissimilarity matrix. Statistically significant differences in taxa abundance were determined using Welch's t-test in the STAMP program. The above statistical tests were all performed using the R vegan package, and the R ggplot2 package was used for plotting (Dixon 2003; Ginestet 2011).

Results

QQ bacteria strain and QSI

Bacterial strain SCSIO 43740 with QQ activity was screened from 75 coral-derived strains (Fig. 2A). The 16S rRNA gene phylogenetic tree indicated that strain SCSIO 43740 belonged to the genus *Pseudoalteromonas* (Fig. 2D). And the 16S rRNA gene sequences of SCSIO 43740 shared 98.11% sequence similarity with *P. piscicida* NBRC 103038 (Fig. 2D). The crude extract of SCSIO 43740 showed distinct QQ activity on the *C. violaceum* CV026 reporter plate (Fig. 2B). Six purified fractions were prepared from the crude extract and sent for QQ bioactivity determination. Compound 3 was identified as the QSI and sent for further chemical structure determination (Fig. 2C).

The ^{1}H NMR data of compound 3 showed the occurrence of two singlets at 1.27 ppm and 1.38 ppm which resemble a di-substituted tertiary butyl group (Fig. S1). Another singlet around 4.85 indicated the

presence of a phenolic hydrogen. The remaining 3 protons were detected in the aromatic downfield region between 6.62 and 7.22 ppm, suggesting a tri-substituted aromatic compound. The data were further supported by ^{13}C NMR (Fig. S2). The ^{13}C NMR spectra of the isolated pure compound exhibited the occurrence of 10 carbon signals, of which 6 were downfield carbon signals between 116.59 and 154.76 ppm located in the aromatic region. The remaining four carbon signals were detected in the upfield region between 30.10 and 35.70 ppm. Out of six carbons, the aromatic substitution was confirmed by the presence of three quaternary carbons at 154.76 ppm, 142.30 ppm, and 136.12 ppm. Moreover, the presence of a phenolic OH group on the quaternary carbon in the most downfield region, 154.76 ppm, was confirmed in the molecule. Among the four carbon signals present in the upfield regions, two were tertiary carbons at 35.7 ppm and 34.98 ppm while the remaining two were methyl signals at 32.15 ppm and 30.10 ppm. The molecular weight was established by quadruple-time-of-flight (Q-TOF) HRMS mass spectrometry, with a mass of [M + H] + at m/z 205.15, corresponding to the major peak, giving an accurate mass of ≈ 206.17, in positive ion mode (Fig. S3). Thus, after interpretation of the hydrogen and carbon spectra, the structure of the compound was structurally elucidated as 2,4-di-tert-butylphenol ($C_{14}H_{22}O$, 2,4-DTBP) (Fig. 2E) (Cho et al. 2016).

Coral physiological response

The physiological response of coral holobiont was estimated by algal symbiont cell abundance (Fig. 3A), PAM fluorometry measurements *Fv/Fm* (Fig. 3B), and the enzyme activities of SOD (Fig. 4A) and CAT (Fig. 4B). For corals maintained at the normal temperature (Con: 29°C), the abundance of coral symbionts ranged between 3.7 $\times 10^6$ and 4.3 $\times 10^6$ cells/ml (Fig. 3A) and showed no significant difference between different time points ($P > 0.05$). In contrast, the high temperature group (HT: 31°C) presented a significant decrease in algal symbiont density of 41% from day 0 to day 30 ($P < 0.05$). However, for high temperature with QQ bacteria inoculation group (HTQQ: 31°C + QQ bacteria), no significant decrease in algal symbiont abundance was observed over time (Fig. 3A). The algal symbiont abundance did not show a statistical difference between the HTQQ group and Con group at day 30 ($P > 0.05$), despite it was slightly lower in the HTQQ group than in the Con group (Fig. 3A). A similar pattern was found for *Fv/Fm* values, where the most obvious change occurred at day 30, with the *Fv/Fm* value in the HT group being significantly lower than that of the Con and HTQQ groups ($P < 0.05$) (Fig. 3B).

Compared to the shift in algal abundance and *Fv/Fm* values, the enzyme activities of SOD and CAT showed a different trend in *P. damicornis* overall. On day 30, both SOD and CAT showed the highest activity in the HTQQ treatment. There was no significant difference in the activities of SOD among the Con group, HT group and HTQQ group at the same time of the experiment. Interestingly, CAT activity showed a similar pattern (Con < HT < HTQQ) among the 3 treatments at each time point (Fig. 4B). The CAT activity was significantly higher in the HTQQ group than in the Con group. However, there was no significant difference in CAT activity between Con and HT or HT and HTQQ at the same time during the experiment (Fig. 4B).

Shift of coral-associated bacteria community

In total, 12,209 OTUs were clustered from 668,568 sequences in 35 coral samples. For alpha diversity of coral microbial communities, the three groups showed no significant difference throughout the experimental period (Supplementary Table 1). In terms of microbial communities, *Proteobacteria*, *Gammaproteobacteria*, *Bacteroidetes*, *Firmicutes*, *Deltaproteobacteria* and *Alphaproteobacteria* dominated all samples in the three treatments (Fig. S4). At day 30, the proportion of *Alphaproteobacteria* decreased to 15.66% in HTQQ and increased to 16.19% in HT (Fig. S4). Further analysis showed that the abundance of ASV252, whose 16S rRNA gene sequence was most similar to SCSIO 43740, increased in the HTQQ group from 0.071–0.303% between day 0 to day 10 and decreased to 0.000% between day 10 to day 30 (Supplementary Table 2).

For beta-diversity, the PCoA plots and ANOSIM analysis indicated no significant difference of the coral-associated bacterial communities was observed among the Con group, HT group and HTQQ group at days 10 and 30 (Fig. 5A and 5B). However, the within-group weighted unifrac dissimilarity of the coral-associated bacterial communities varied significantly among different treatments (Fig. 5C). The coral microbial communities in the HT group showed the highest within-group weighted unifrac dissimilarity at both day 10 and day 30. The beta dispersion of coral microbial communities as indicated by weighted unifrac dissimilarity in the HT group was significantly higher than that of the control group ($P < 0.05$), but for the HTQQ group with QQ bacterial inoculation, there was no significant difference compared to the control group regarding the beta dispersion of coral microbial communities (Fig. 5C).

A total of 15 ASVs showing significant differences between HT and HTQQ groups at day 30 were identified as bioindicators (Fig. S5). Most of the ASVs classified as bioindicators belonged to *Proteobacteria* (8 ASVs), followed by *Cyanobacteria* (4 ASVs) (Fig. S5). ASVs belonging to *Acidaminobacter* and *Stappia* were the most dominant ASVs present in the HT group (Fig. S5). ASV127 and ASV272, classified as *Chroobacteria*, were the most dominant and enriched indicators in the HTQQ group on day 30.

Response of co-occurrence network

To explore the shift in microbial community, a random matrix theory-based method was employed to construct a co-occurrence network with complex visual pictures (Fig. 6A). The networks of the Con, HT and HTQQ groups contained 81, 84 and 87 nodes, respectively (Table S3). The HTQQ network had more interactions than the other networks. A total of 1 connector was detected in the Con network and 1 module hub was detected in the HT network, while there were 18 connectors in the HTQQ network (Fig. 6B). The detailed taxonomic properties of keystone taxa in bacterial networks were listed in Table S4. According to the Zi-Pi plot, *Deltaproteobacteria* and *Alphaproteobacteria*, were abundant nodes, also acted as the keystone taxa in the bacterial network (Fig. 6B and Table S4).

The microbial network stability was tested by simulating taxon extinction and calculating the natural connectivity (Fig. 6C). We found that the robustness of the Con microbial network was the highest. And it in HTQQ microbial network was higher than that of HT microbial network, showing a higher network stability of HTQQ. In addition, the natural connectivity of the microbial networks decreased to a greater

degree with a greater fluctuation in HT than in the HTQQ by removing the same number of nodes, indicating a higher stability of the HTQQ network.

Discussion

QQ & QSI in corals

The coral holobiont is a rich repository of QQ&QSI. Researchers have isolated many QQ bacterial isolates in coral symbiotic microorganisms, including *Vibrio sp.*, *Delftia sp.*, *Lysinibacillus sp.*, *Staphylococcus sp.*, *Bacillus sp.* and so on (Bakkiyaraj et al. 2013; El-Kurdi et al. 2021; Ma et al. 2018; Song et al. 2018). In this work, a QQ bacterium belonging to *Pseudoalteromonas sp*. was found from *P. damicornis*. Previous studies have reported QQ bacterial strains with AHLs-degrading function (e.g., *P. tetraodonis* strain MQS005, *P. flavipulchra* JG1, etc.) belonging to the genus *Pseudoalteromonas* sp. (Linthorne et al. 2015; Liu et al. 2017; Pan et al. 2019). Some studies have also isolated effective QSI compounds from corals, such as DL-homocysteine thiolactone (Ma et al. 2018) and rhodamine isothiocyanate (Song et al. 2018) (summarized in Table S5). This study is the first report of coral-derived *Pseudomonas* bacterial strain producing the QSI compound 2,4-DTBP (Fig. 2E). It increases the resources available for coral reef conservation via manipulation of microbial QS related process.

QQ bacteria and QSI compounds are potential tools for coral reef conservation. Studies have shown that QSI compounds (e.g., furanone) can reduce the white belt disease and stabilize bacterial communities in corals (Certner and Vollmer 2018). Although the QSI chemical furanone has shown good performance in improving coral health (Certner and Vollmer 2018; Sun et al. 2023; Zhou et al. 2020), the application of QSI in mitigating coral bleaching is still at the preliminary exploration stage. Many QSI compounds isolated from QQ bacteria, including 2,4-DTBP, have not been tested for their potential effects in alleviating coral bleaching. Therefore, further experiments exploring the effects and mechanisms of coral bleaching mitigation by QQ bacteria and QSI compounds will broaden the field of coral microbial remediation. The 2,4-DTBP obtained in this study can competitively bind to the QS receptors LasR and Rh1R with C4-HSL, and regulate the expression of virulence phenotypes (Mishra et al. 2020; Pattnaik et al. 2018). In this work, only *C. violaceum* CV026, which can produce purple pigment in direct response to the chemical signals C4-HSL and C6-HSL (McClean et al. 1997), was adopted to screen QQ bacteria. Some QQ mechanisms via other pathways are also worth trying to screen QQ bacteria and QSI compounds from corals in the future, such as the DPD or CAI-1-mediated signal molecule communication system (Surette and Bassler 1998; Swift et al. 1997; Winson et al. 1998).

QQ & QSI alleviating thermal stress

Under global climate change, mitigating coral bleaching is one of the urgent problems to be solved (Hughes et al. 2017). Inoculation of BMCs consortium into corals has been shown to be an effective way to mitigate coral thermal bleaching (Rosado et al. 2019; Santoro et al. 2021). Rosado et al (2019) was the first to inoculate corals with bacterial consortia, and found a significant reduction in coral-bleaching metrics under thermal stress (Rosado et al. 2019). Santoro et al (2021) found that BMC inoculation led

to microbiome reorganization, which altered coral genetics and metabolism, ultimately reducing coral bleaching and mortality (Santoro et al. 2021). A study also shown that transplantation of microbiome from thermal-tolerant corals can alleviate thermal bleaching in the short term (Doering et al. 2021). However, these studies used a mixture of bacterial consortia and the specific compounds that are effective in mitigating coral bleaching remains unclear.

Under elevated seawater temperature, coral holobionts produce more signaling molecules, such as *Cholerae* autoinducer-1 (CAI-1), autoinducer-2 (AI-2) and AHLs, which may attract pathogenic bacteria and increase infection risks (Bhedi et al. 2017; Kimes et al. 2012). It is proposed that enhanced QS signaling molecules triggering pathogenic processes may disrupt the ecological balance of coral symbionts and contribute to coral bleaching (Zhou et al. 2020). Previous studies have shown that the addition of either QQ bacteria or QSI can reduce pathogenic and bleaching damage under normal temperatures (Torres et al. 2016; Zhou et al. 2020). This study found that inoculation with QQ bacteria can alleviate thermal stress-induced photosynthetic physiological impairment in the coral *P. damicornis* (Fig. 3). This research is the first attempt to apply QQ bacteria inoculation to mitigate coral bleaching under thermal stress, and provides a new strategy for coral microbiome engineering.

Under elevated temperatures, the changes in coral cell membrane fluidity can increase the generation of ROS, leading to further exocytosis of symbiont algae and cell death (Lesser 2006). One study has found that ROS can be managed by CAT and SOD from coral symbionts during thermal stress (Gardner et al. 2017). In this study, SOD and CAT content in corals at the endpoint were observed an increased activity after adding QQ bacteria (HTQQ group) compared to the high temperature treatment (HT group) (Fig. 4). It indicates that inoculating QQ bacteria SCSIO 43740 may also act indirectly by enhancing the ROS scavenging capacity of coral symbionts.

QQ bacteria improve the stability of the coral microbiome

Environmental perturbations such as high temperatures can cause microbial communities to shift from stable to unstable states (Zaneveld et al. 2017). The coral *Mussismilia hispida* showed significant changes in bacterial community structure and stability under 30°C warming stress (Santoro et al. 2021). However, after inoculation with BMCs *M. hispida* microbiome showed a more stable microbial communities under thermal stress (Santoro et al. 2021). In our study, the bacterial community of *P. damicornis* also showed similar trends after inoculation with QQ bacteria, with the beta-dispersion of HTQQ group being significantly lower than that of the HT group (Fig. 5C). According to the Anna Karenina hypothesis, disorganized or stressed organisms have more diverse and unstable microbial communities than healthy individuals (Zaneveld et al. 2017). The addition of QQ bacterium SCSIO 43740 can mitigate the turbulence of coral microbial community under thermal stress, which is critical for maintaining the health of the coral holobiont. Similar to the previous study (Rosado et al. 2019), the inoculated QQ bacteria SCSIO 43740 was not detected in all samples by sequencing analysis (Table. S2). This might be related to the sampling time windows. As their colonization and activity in corals remains unclear, the mechanisms of how QQ bacteria function to alleviate thermal stress in corals need to be further investigated.

In recent years, microbial co-occurrence networks have been used to better understand microbial interactions within a community (Yuan et al. 2021; Zhu et al. 2023). Simple networks with low connectivity are less robust than complex ones in the face of external perturbations (Santolini and Barabasi 2018). In this study, the highest connectors and complex interactions occurred in the HTQQ group, while the loosest connectivity occurred in the HT group (Fig. 6). Furthermore, the natural connectivity of the network also showed that the network robustness of the HTQQ group was higher than that of HT group (Fig. 6C). The shift pattern of the coral microbiome stability indicated by both community beta-dispersion and network robustness is consistent. This study shows that QQ bacteria may exert a stronger influence on the microbial structure and co-occurrence pattern to maintain its stability, and may eventually be involved in reducing the occurrence of heat-induced coral bleaching.

Conclusions

In this study, *Pseudoalteromonas* SCSIO 43740 from *Pocillopora damicornis* was screened, and it is the first report of *Pseudoalteromonas* derived QSI 2,4-DTBP in corals. Inoculation with QQ bacteria alleviated thermal bleaching stress in corals at elevated water temperature. The main probiotic effects observed included the maintenance of photosynthetic efficiency, the enhancement of ROS scavenging related enzyme activities, and the stabilization of microbial community interactions. This study lays a solid foundation for the development of coral microbial engineering based on the QQ principle, and provides potential new tools for coral reef ecological conservation in a warming ocean. Further research investigating the functioning mechanisms can be carried out by tracking the colonization and activity of inoculated QQ bacteria in the coral host. New technologies such as quantitative full-length 16S rRNA sequencing, in situ fluorescence tracing, enzymological and metabolomic methods might be helpful to understand the probiotic mechanism of inoculated QQ bacteria in the coral holobiont in the future studies.

Declarations

Author contributions

Conceptualization, Junde Dong and Juan Ling; Experiment and Data Analysis, Qingsong Yang Bin Yang, Wenqian Zhang, Xiaoyu Tang and Huiming Sun; Draft Preparation, Bing Yang and Qingsong Yang; Bin Yang, Yanying Zhang, and Jie Li revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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Availability of Data and Materia

The sequencing data were deposited in the NCBI Sequence Read Archive database, with accession number PRJNA983116.

Ethics Approval and Consent to Participate

Not applicable.

Conflict of Interest

The authors declare no competing interests.

References

1. Anthony KRN (2016) Coral Reefs Under Climate Change and Ocean Acidification: Challenges and Opportunities for Management and Policy. Annual Review of Environment and Resources. Annu Rev Environ Resour 41:59-81
2. Bakkiyaraj D, Sivasankar C, Pandian SK (2013) Anti-pathogenic potential of coral associated bacteria isolated from gulf of mannar against *pseudomonas aeruginosa*. Indian J Microbiol 53:111-113
3. Banin E, Vassilakos D, Orr E et al (2003) Superoxide dismutase is a virulence factor produced by the coral bleaching pathogen *vibrio shiloi*. Curr Microbiol 46:418-422
4. Bauer A, Kirby W, Sherris J et al (1966) Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 45:493-496
5. Berry D, Widder S (2014) Deciphering microbial interactions and detecting keystone species with co-occurrence networks. Front Microbiol 5:219
6. Bhedi CD, Prevatte CW, Lookadoo MS et al (2017) Elevated temperature enhances short- to medium-chain acyl homoserine lactone production by black band disease-associated vibrios. FEMS Microbiol Ecol 93:3
7. Bolyen E, Rideout JR, Dillon MR et al (2019) Author correction: Reproducible, interactive, scalable and extensible microbiome data science using qiime 2. Nat Biotechnol 37:1091
8. Bourne DG, Morrow KM, Webster NS (2016) Insights into the coral microbiome: Underpinning the health and resilience of reef ecosystems. Annu Rev Microbiol 70:317-340
9. Callahan BJ, McMurdie PJ, Rosen MJ et al (2016) Dada2: High-resolution sample inference from illumina amplicon data. Nat Methods 13:581-583
10. Carlson RR, Evans LJ, Foo SA et al (2021) Synergistic benefits of conserving land-sea ecosystems. Glob Ecol Conserv 28:e01684

11. Certner RH, Vollmer SV (2018) Inhibiting bacterial quorum sensing arrests coral disease development and disease-associated microbes. Environ Microbiol 20:645-657
12. Chen PY, Chen CC, Chu L et al (2015) Evaluating the economic damage of climate change on global coral reefs. Glob Environ Change-Human Policy Dimens 30:12-20
13. Cho JY, Kim MS, Lee YG et al (2016) A phenyl lipid alkaloid and flavone c-diglucosides from spergularia marina. Food Sci Biotechnol 25:63-69
14. Cole JR, Wang Q, Cardenas E et al (2009) The ribosomal database project: Improved alignments and new tools for rrna analysis. Nucleic Acids Res 37:D141-D145
15. Dixon P (2003) Vegan, a package of r functions for community ecology. J Veg Sci 14:927-930
16. Doering T, Wall M, Putchim L et al (2021) Towards enhancing coral heat tolerance: A "microbiome transplantation" treatment using inoculations of homogenized coral tissues. Microbiome 9:102
17. Eakin CM, Sweatman HP A, Brainard RE (2019) The 2014-2017 global-scale coral bleaching event: Insights and impacts. Coral Reefs 38:539-545
18. El-Kurdi N, Abdulla H, Hanora A (2021) Anti-quorum sensing activity of some marine bacteria isolated from different marine resources in egypt. Biotechnol Lett 43:455-468
19. Gardner SG, Raina JB, Nitschke MR et al (2017) A multi-trait systems approach reveals a response cascade to bleaching in corals. BMC Biol 15:117
20. Garren M, Son K, Raina JB et al (2014) A bacterial pathogen uses dimethylsulfoniopropionate as a cue to target heat-stressed corals. ISME J 8:999-1007
21. Ginestet C (2011) Ggplot2: Elegant graphics for data analysis. J R Stat Soc Ser A-Stat. Soc 174:245-245
22. Grottoli AG, Rodrigues LJ, Palardy JE (2006) Heterotrophic plasticity and resilience in bleached corals. Nature 440:1186-1189
23. Haas AF, Guibert M, Foerschner A et al (2015) Can we measure beauty? Computational evaluation of coral reef aesthetics. PeerJ 3:e1390
24. Hill CE L, Lymperaki MM, Hoeksema BW (2021) A centuries-old manmade reef in the caribbean does not substitute natural reefs in terms of species assemblages and interspecific competition. Mar Pollut Bull 169:112576
25. Hughes TP, Kerry JT, Alvarez-Noriega M et al (2017) Global warming and recurrent mass bleaching of corals. Nature 543:373-377
26. ICRI G, Australia Institute of Marine Science, UNEP (2021) Status of coral reefs of the world: 2020
27. Kimes NE, Grim CJ, Johnson WR et al (2012) Temperature regulation of virulence factors in the pathogen vibrio coralliilyticus. ISME J 6:835-846
28. Larkin MA, Blackshields G, Brown NP et al (2007) Clustal w and clustal x version 2.0. Bioinformatics 23:2947-2948
29. Lesser MP (2006) Oxidative stress in marine environments: Biochemistry and physiological ecology. Annu Rev Physiol 68:253-278

30. Linthorne JS, Chang BJ, Flematti GR et al (2015) A direct pre-screen for marine bacteria producing compounds inhibiting quorum sensing reveals diverse planktonic bacteria that are bioactive. Mar Biotechnol (NY) 17:33-42
31. Liu N, Yu M, Zhao Y et al (2017) Pfma, a novel quorum-quenching *n*-acylhomoserine lactone acylase from *pseudoalteromonas flavipulchra*. Microbiology 163:1389-1398
32. Ma ZP, Song Y, Cai ZH et al (2018) Anti-quorum sensing activities of selected coral symbiotic bacterial extracts from the south china sea. Front Cell Infect Microbiol 8:144
33. McClean KH, Winson MK, Fish L et al (1997) Quorum sensing and chromobacterium violaceum: Exploitation of violacein production and inhibition for the detection of n-acylhomoserine lactones. Microbiology 143:3703-3711
34. McLean RJ, Pierson LS, 3rd, Fuqua C (2004) A simple screening protocol for the identification of quorum signal antagonists. J Microbiol Methods 58:351-360
35. Mishra R, Kushveer JS, Khan MIK et al (2020) 2,4-di-tert-butylphenol isolated from an endophytic fungus, daldinia eschscholtzii, reduces virulence and quorum sensing in pseudomonas aeruginosa. Front Microbiol 11:1668
36. Montesinos-Navarro A, Hiraldo F, Tella JL et al (2017) Network structure embracing mutualism-antagonism continuums increases community robustness. Nat Ecol Evol 1:1661-1669
37. Morrison TH, Hughes TP, Adger WN et al (2019) Save reefs to rescue all ecosystems. Nature 573:333-336
38. Nesa B, Hidaka M (2009) High zooxanthella density shortens the survival time of coral cell aggregates under thermal stress. J Exp Mar Biol Ecol 368:81-87
39. Nithya C, Begum MF, Pandian SK (2010) Marine bacterial isolates inhibit biofilm formation and disrupt mature biofilms of pseudomonas aeruginosa pao1. Appl Microbiol Biotechnol 88:341-358
40. Pan Y, Wang Y, Yan X et al (2019) Quorum quenching enzyme aptm01, an acylhomoserine-lactone acylase from marine bacterium of pseudoalteromonas tetraodonis strain mqs005. Curr Microbiol 76:12
41. Pattnaik SS, Ranganathan S, Ampasala DR et al (2018) Attenuation of quorum sensing regulated virulence and biofilm development in pseudomonas aeruginosa pao1 by diaporthe phaseolorum ssp12. Microb Pathog 118:177-189
42. Paulson JN, Stine OC, Bravo HC et al (2013) Differential abundance analysis for microbial marker-gene surveys. Nat Methods 10:1200
43. Peixoto RS, Rosado PM, Leite DC et al (2017) Beneficial microorganisms for corals (bmc): Proposed mechanisms for coral health and resilience. Front Microbiol 8:341
44. Peixoto RS, Sweet M, Villela HD M et al (2021) Coral probiotics: Premise, promise, prospects. Annual Review of Animal Biosciences. 9:265-288
45. Petersen LE, Kellermann MY, Schupp PJ (2020) Secondary metabolites of marine microbes: From natural products chemistry to chemical ecology. YOUMARES 9 - THE OCEANS: OUR RESEARCH, OUR

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46. Petersen LE, Moeller M, Versluis D et al (2021) Mono- and multispecies biofilms from a crustose coralline alga induce settlement in the scleractinian coral leptastrea purpurea. Coral Reefs 40:381-394
47. Raina JB, Tapiolas DM, Foret S et al (2013) Dmsp biosynthesis by an animal and its role in coral thermal stress response. Nature 502:677-680
48. Ralph PJ, Schreiber U, Gademann R et al (2005) Coral photobiology studied with a new imaging pulse amplitude modulated fluorometer. J Phycol. 41:335-342
49. Rosado PM, Leite DC A, Duarte GAS et al (2019) Marine probiotics: Increasing coral resistance to bleaching through microbiome manipulation. ISME J 13:921-936
50. Rosenberg E, Koren O, Reshef L et al (2007) The role of microorganisms in coral health, disease and evolution. Nat Rev Microbiol 5:355-362
51. Santolini M, Barabasi A-L (2018) Predicting perturbation patterns from the topology of biological networks. Proc Natl Acad Sci U S A 115:E6375-E6383
52. Santoro EP, Borges RM, Espinoza JL et al (2021) Coral microbiome manipulation elicits metabolic and genetic restructuring to mitigate heat stress and evade mortality. Sci Adv 7:33
53. Shannon P, Markiel A, Ozier O et al (2003) Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Res 13:2498-2504
54. Sharp KH, Sneed JM, Ritchie KB et al (2015) Induction of larval settlement in the reef coral porites astreoides by a cultivated marine roseobacter strain. Biol Bull 228:2
55. Silva DP, Villela HD M, Santos HF et al (2021) Multi-domain probiotic consortium as an alternative to chemical remediation of oil spills at coral reefs and adjacent sites. Microbiome 9:118
56. Song Y, Cai ZH, Lao YM et al (2018) Antibiofilm activity substances derived from coral symbiotic bacterial extract inhibit biofouling by the model strain pseudomonas aeruginosa pao1. Microb Biotechnol 11:1090-1105
57. Stambler N (2011) Zooxanthellae: The yellow symbionts inside animals. Coral Reefs: An Ecosystem in Transition Springer Netherlands 87-106
58. Sun X, Li Y, Yang Q et al (2023) Identification of quorum sensing-regulated vibrio fortis as potential pathogenic bacteria for coral bleaching and the effects on the microbial shift. Front Microbiol 14:1116737
59. Surette MG, Bassler BL (1998) Quorum sensing in *escherichia coli* and *salmonella typhimurium*. Proc Natl Acad Sci U S A 95:7046-7050
60. Swift S, Karlyshev AV, Fish L et al (1997) Quorum sensing in *aeromonas hydrophila* and *aeromonas salmonicida*: Identification of the luxri homologs ahyri and asari and their cognate *n*-acylhomoserine lactone signal molecules. J Bacteriol 179:5271-5281
61. Tait K, Hutchison Z, Thompson FL et al (2010) Quorum sensing signal production and inhibition by coral-associated vibrios. Environ Microbiol Rep 2:145-150

62. Tamura K, Stecher G, Kumar S (2021) Mega11: Molecular evolutionary genetics analysis version 11. Mol Biol Evol 38:3022-3027
63. Torres M, Rubio-Portillo E, Anton J et al (2016) Selection of the n-acylhomoserine lactone-degrading bacterium alteromonas stellipolaris pqq-42 and of its potential for biocontrol in aquaculture. Front Microbiol 7:646
64. Wang T, Cheng KK, Cai ZH et al (2022) Research advances in communication interactions among the symbionts of "bacteria-zooxanthellae-coral". J Appl Ecol 33:2572-2584
65. Weisburg WG, Barns SM, Pelletier DA et al (1991) 16s ribosomal DNA amplification for phylogenetic study. J Bacteriol 173:697-703
66. Welsh RM, Rosales SM, Zaneveld JR et al (2017) Alien vs. Predator: Bacterial challenge alters coral microbiomes unless controlled by halobacteriovorax predators. PeerJ 5:e3315
67. Winson MK, Swift S, Fish L et al (1998) Construction and analysis of *luxcdabe*-based plasmid sensors for investigating *n*-acyl homoserine lactone-mediated quorum sensing. FEMS Microbiol Lett 163:185-192
68. Wu Y, Zhou Z, Wang J et al (2019) Temperature regulates the recognition activities of a galectin to pathogen and symbiont in the scleractinian coral pocillopora damicornis. Dev Comp Immunol 96:103-110
69. Yoon SH, Ha SM, Kwon S et al (2017) Introducing ezbiocloud: A taxonomically united database of 16s rrna gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol 67:1613-1617
70. Yuan MM, Guo X, Wu L et al (2021) Climate warming enhances microbial network complexity and stability. Nat Clim Chang 11:343-348
71. Zaneveld JR, McMinds R, Vega Thurber R (2017) Stress and stability: Applying the anna karenina principle to animal microbiomes. Nat Microbiol 2:17121
72. Zhang Y, Yang Q, Ling J et al (2021a) Shifting the microbiome of a coral holobiont and improving host physiology by inoculation with a potentially beneficial bacterial consortium. BMC Microbiol 21:130
73. Zhang Y, Yang Q, Zhang Y et al (2021b) Shifts in abundance and network complexity of coral bacteria in response to elevated ammonium stress. Sci Total Environ 768:144631
74. Zhou J, Lin ZJ, Cai ZH et al (2020) Opportunistic bacteria use quorum sensing to disturb coral symbiotic communities and mediate the occurrence of coral bleaching. Environ Microbiol 22:1944-1962
75. Zhu W, Liu X, Zhu M et al (2023) Coastal upwelling under anthropogenic influence drives the community change, assembly process, and co-occurrence pattern of coral associated microorganisms. J Geophys Res-Oceans 128:2

Figures

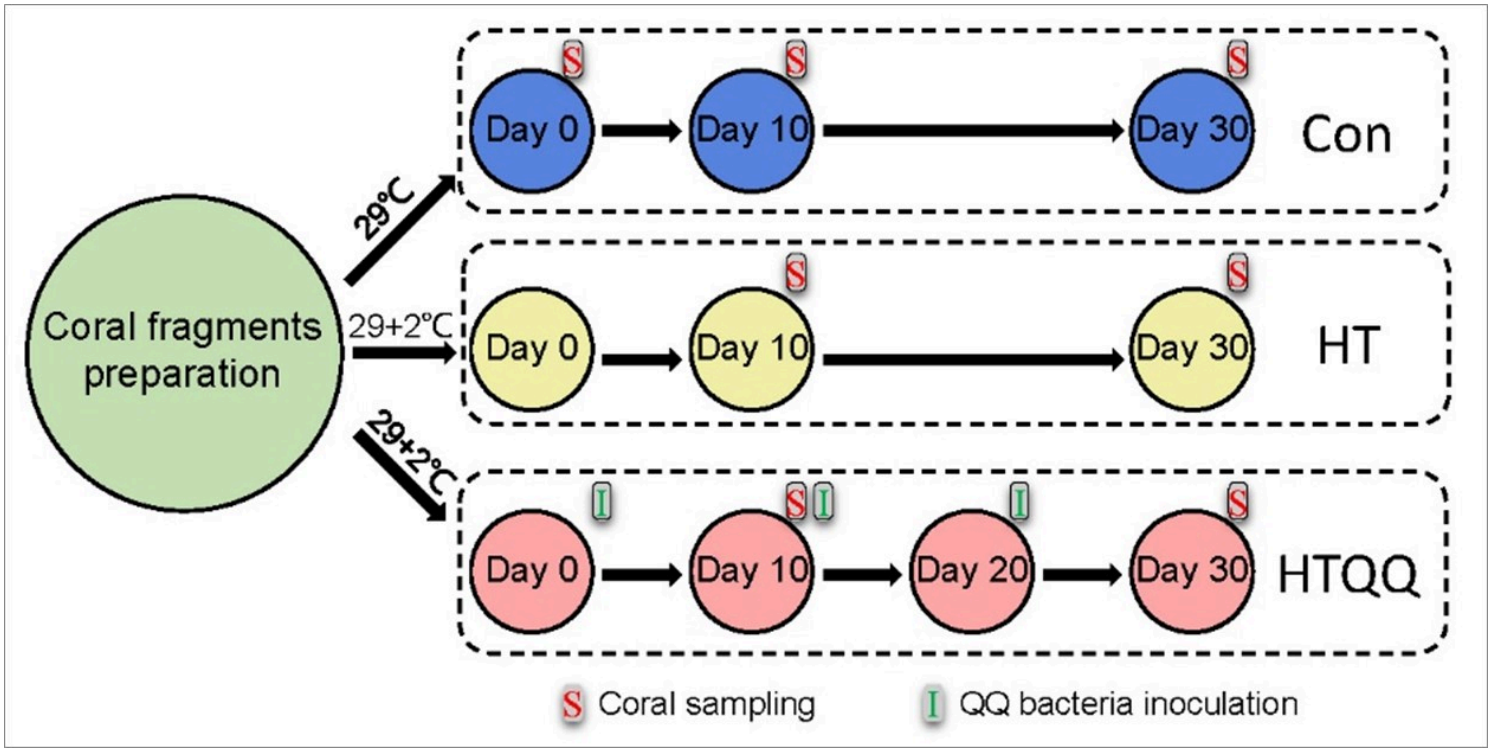


Figure 1

The schematic of the experimental design. Three treatments including control group (Con: 29℃), high temperature group (HT: 31℃), and the high temperature with QQ bacteria inoculation group (HTQQ: 31℃+QQ bacteria) were set up in the experiment. Five coral fragments were collected on day 0, 10, and 30 respectively.

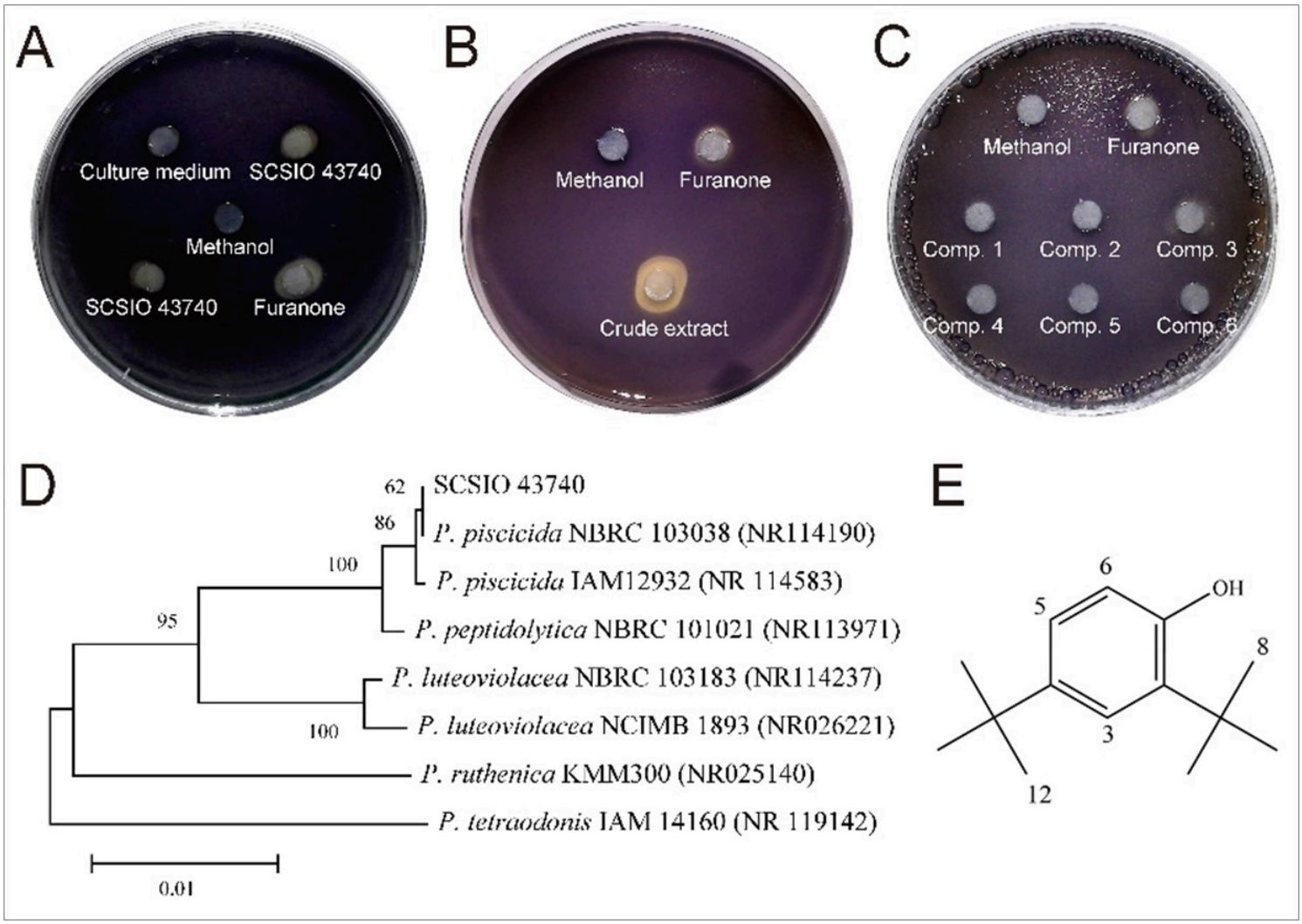


Figure 2

Screening and identification of QQ bacterial strain and its QSI molecular. The QQ activity test of bacterial strain **(A)**, crude extract **(B)**and pure components **(C)** on biosensor plates containing reference strain CV026. Methanol and furanone (dissolved in methanol) were used as negative and positive controls, respectively. The inhibition of purple pigment formation indicates potential QSI activity. **(D)** Unrooted neighbor-joining phylogenetic tree based on the full-length 16S rRNA gene of QQ bacterium SCSIO 43740 and its relatives. The bootstrap values (> 50%) of relevant nodes are shown based on 1000 replicates. **(E)** Structures of the QSI compounds isolated from the QQ bacterium *Pseudoalteromonas piscicida* SCSIO 43740.

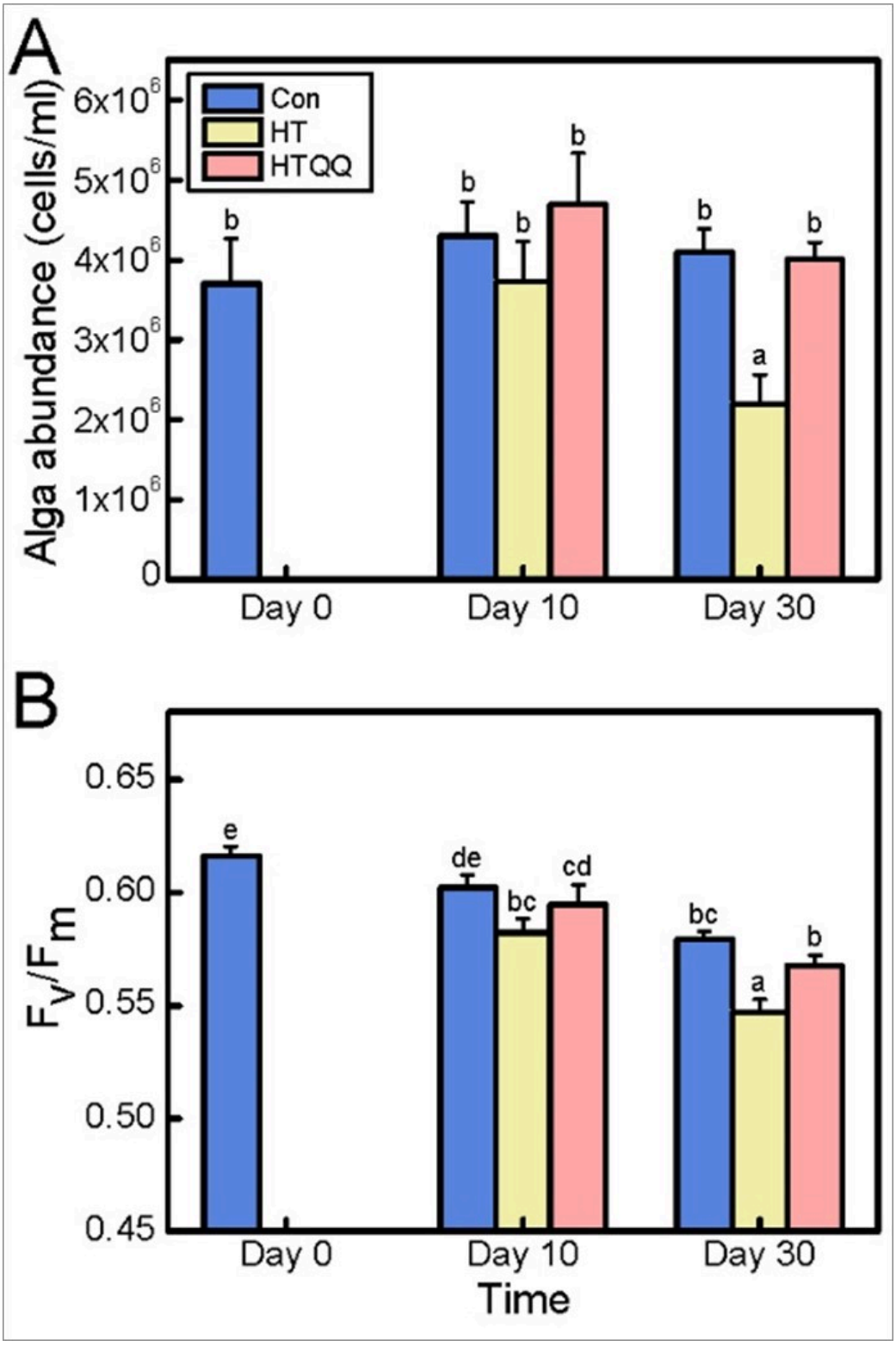


Figure 3

Photosynthetic physiology of coral *Pocillopora damicornis* in each treatment. Symbionts algal abundance **(A)** and *Fv/Fm* ratio **(B)**were measured at each time point. Multiple comparisons were analyzed using LSD test and different letters indicate $P<0.05$.

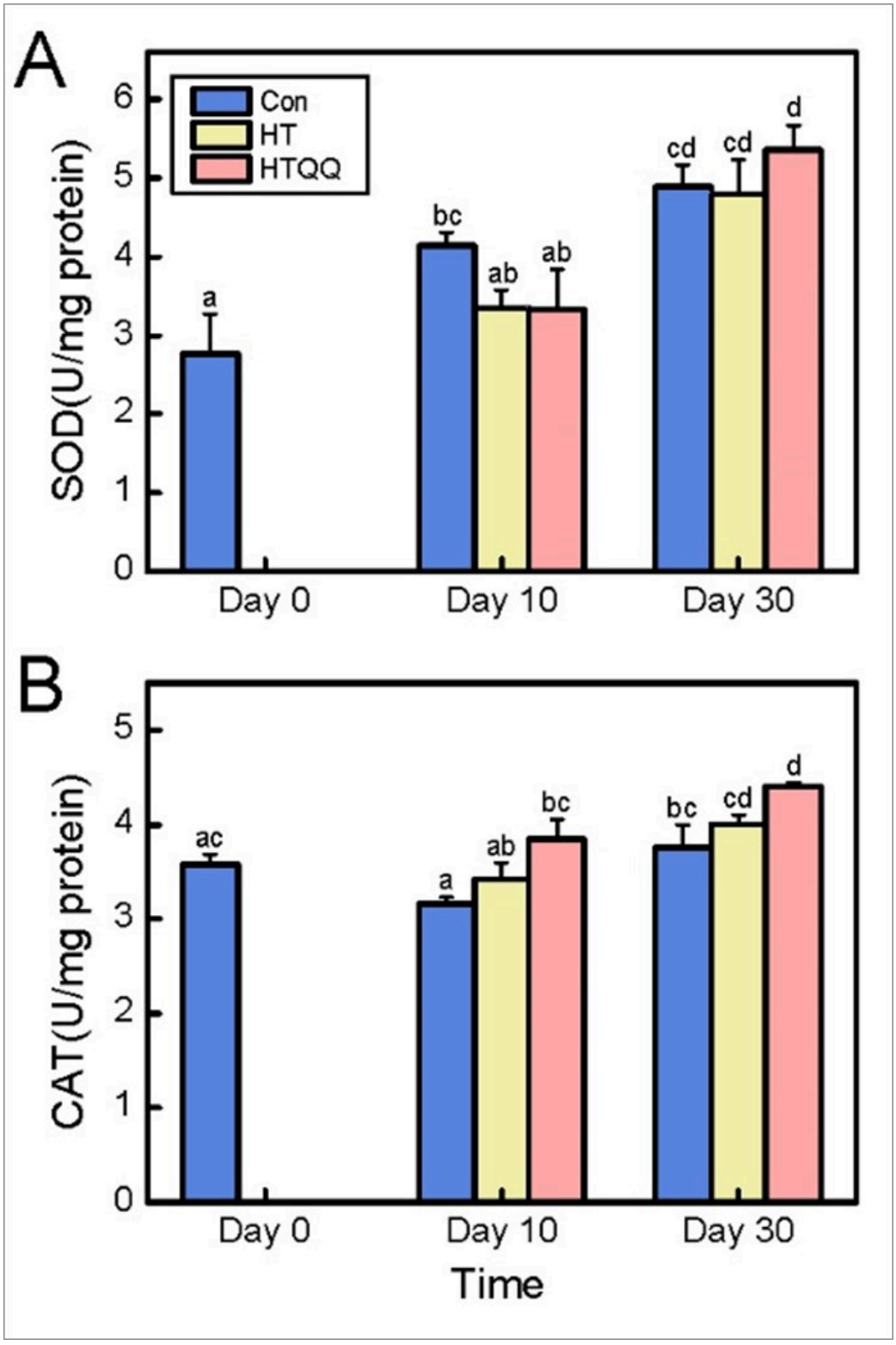


Figure 4

Antioxidant enzymatic activity of coral *Pocillopora damicornis* in each treatment. Superoxide dismutase (SOD) **(A)** and catalase (CAT) **(B)** were measured at each time point. Multiple comparisons were analyzed using LSD test and different letters indicate $P<0.05$.

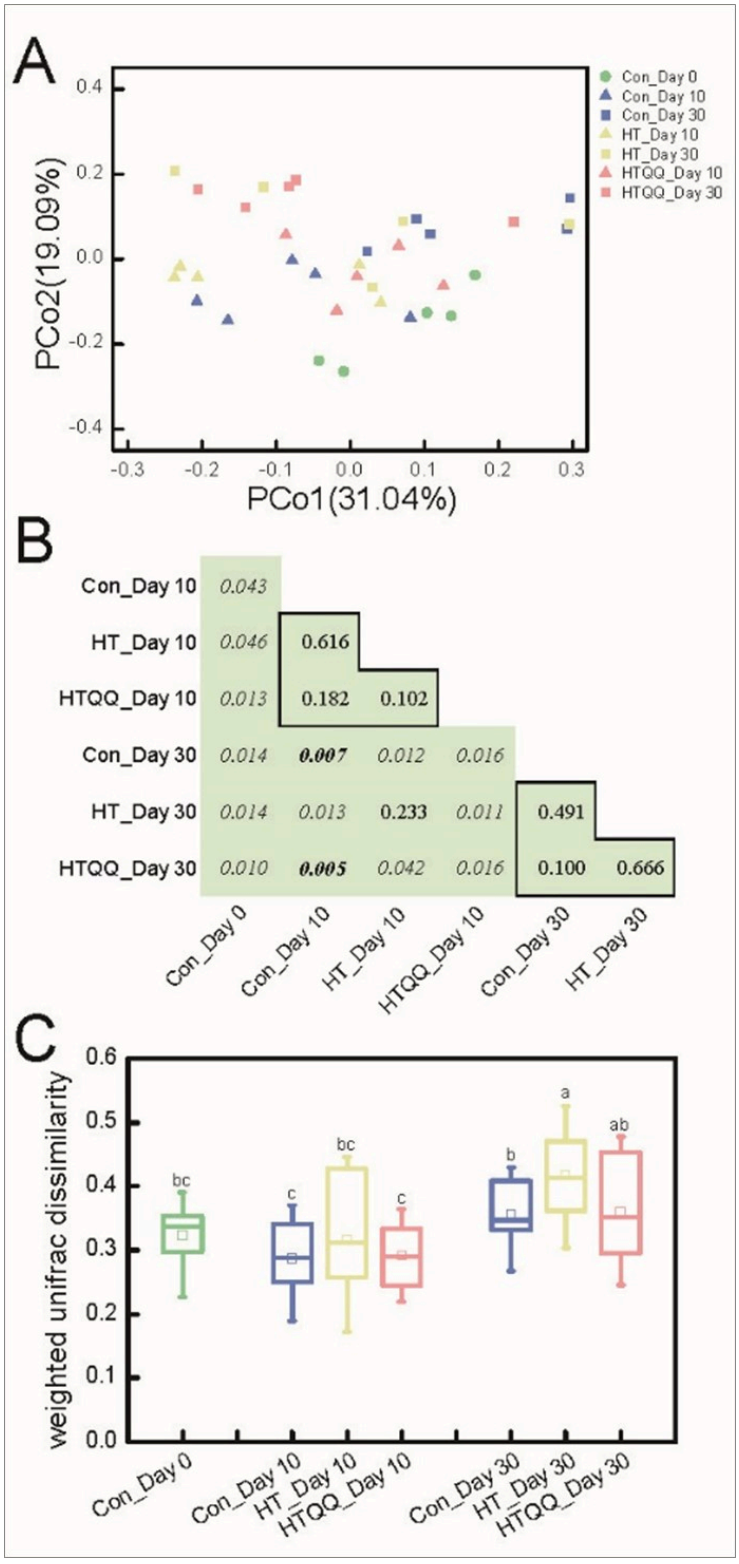


Figure 5

Beta diversity of coral-associated bacterial communities. (A) PCoA plot based on the weighted unifrac distance matrix of coral-associated bacterial communities at ASVs level. **(B)** Pair-wise *P*-values of the ANOSIM of bacterial community composition at ASVs level based on the weighted unifrac distance matrix. **(C)** The within-group weighted unifrac dissimilarity of the coral-associated bacterial communities. Multiple comparisons were analyzed using LSD test and different letters indicate $P<0.1$.

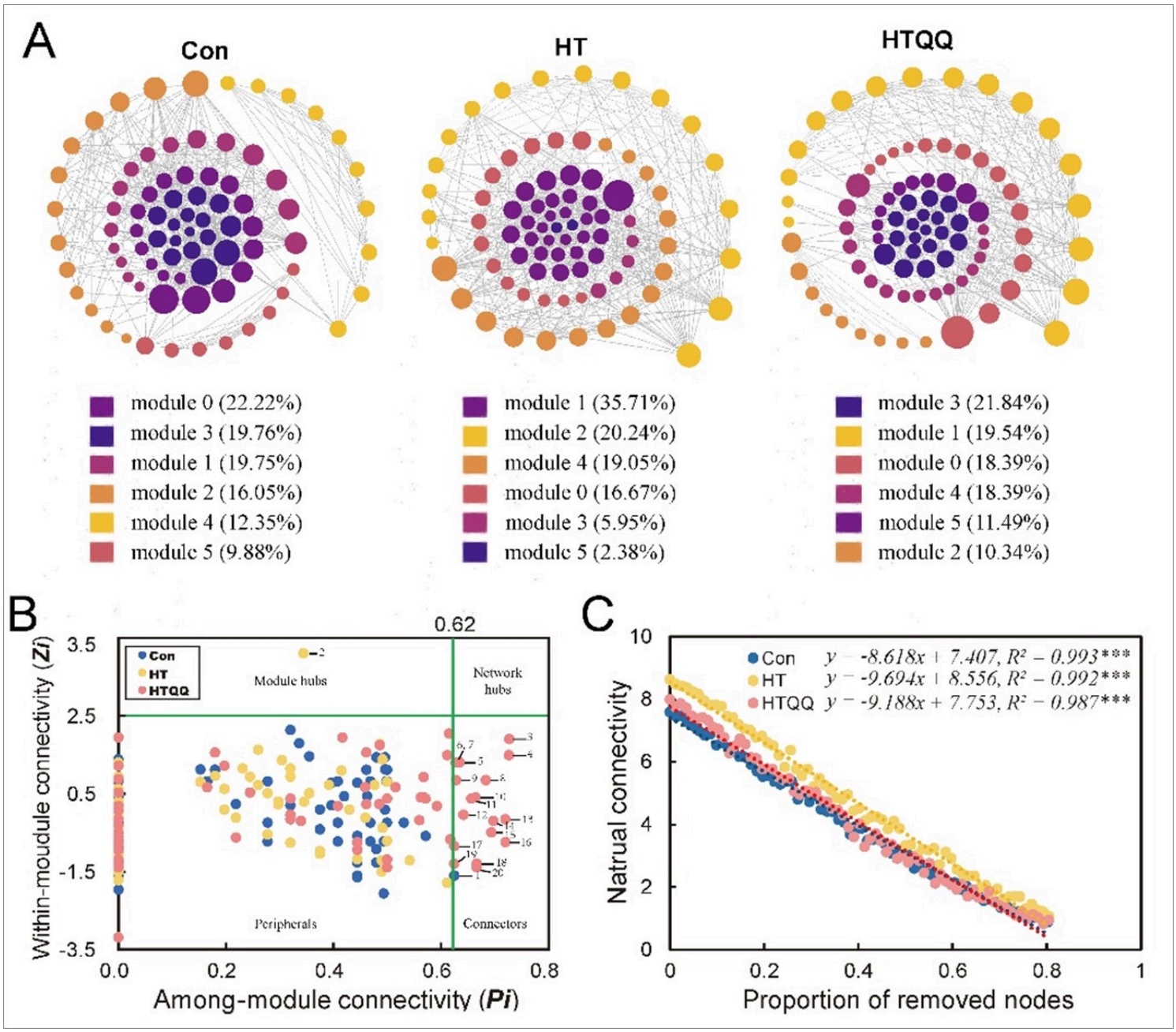


Figure 6

The co-occurrence network analysis of coral associated bacterial communities. (A) The bacterial network structure and module analysis of three treatments. **(B)** *Zi-Pi* plots showed the topological role of each node (*Zi* > 2.5, *Pi* > 0.62). **(C)** Robustness was shown as relationships between number of removed nodes and natural connectivity of the network, such that a larger slope indicates less robustness. *** *P*< 0.001.

Supplementary Files

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