BMC Genomics

RESEARCH Open Access

A transcriptome-wide analysis provides novel insights into how *Metabacillus indicus* promotes coral larvae metamorphosis and settlement

Yanying Zhang[1*†], Ying Zhang[2†], Xiaoyu Tang[2], Xiangrui Guo[1], Qingsong Yang[2,4], Hao Sun[1], Hanzhang Wang[1], Juan Ling[2,4] and Junde Dong[2,3,4*]

Abstract

Background Coral reefs experience frequent and severe disturbances that can overwhelm their natural resilience. In such cases, ecological restoration is essential for coral reef recovery. Sexual reproduction has been reported to present the simplest and most cost-effective means for coral reef restoration. However, larval settlement and post-settlement survival represent bottlenecks for coral recruitment in sexual reproduction. While bacteria play a significant role in triggering coral metamorphosis and settlement in many coral species, the underlying molecular mechanisms remain largely unknown. In this study, we employed a transcriptome-level analysis to elucidate the intricate interactions between bacteria and coral larvae that are crucial for the settlement process.

Results High *Metabacillus indicus* strain cB07 inoculation densities resulted in the successful induction of metamorphosis and settlement of coral *Pocillopora damicoris* larvae. Compared with controls, inoculated coral larvae exhibited a pronounced increase in the abundance of strain cB07 during metamorphosis and settlement, followed by a significant decrease in total lipid contents during the settled stage. The differentially expressed genes (DEGs) during metamorphosis were significantly enriched in amino acid, protein, fatty acid, and glucose related metabolic pathways. In settled coral larvae induced by strain cB07, there was a significant enrichment of DEGs with essential roles in the establishment of a symbiotic relationship between coral larvae and their symbiotic partners. The photosynthetic efficiency of strain cB07 induced primary polyp holobionts was improved compared to those of the negative controls. In addition, coral primary polyps induced by strain cB07 showed significant improvements in energy storage and survival.

†Yanying Zhang and Ying Zhang contributed equally to this work.

*Correspondence:
Yanying Zhang
zhyanying@ytu.edu.cn
Junde Dong
dongjunde@vip.163.com

Full list of author information is available at the end of the article

Conclusions Our findings revealed that strain cB07 can promote coral larval settlement and enhance post-settlement survival and fitness. Manipulating coral sexual reproduction with strain cB07 can overcome the current recruitment bottleneck. This innovative approach holds promise for future coral reef restoration efforts.

Keywords Bacteria, Coral, Metamorphosis, Settlement, Transcriptome

Background

Coral reefs are highly productive and diverse ecosystems. They support the livelihoods of millions of people worldwide by affording coastal protection and serving as an important source of food and pharmaceuticals, in addition to possessing substantial recreational and touristic value [1, 2]. Globally, the biodiversity and function of coral reefs have been declining due to both natural and anthropogenic stressors [3–5]. However, natural reef recovery is slow, even under optimal conditions [6, 7]. Over recent years, attempts at coral reef restoration have focused on minimizing coral loss and enhancing coral recovery by both asexual and sexual reproduction. Regarding the former, it is common practice to fragment or microfragment corals and then plant them in artificial nurseries to produce coral fragments that can be outplanted onto degraded reefs [8, 9]. The latter, meanwhile, represents a sustainable and cost-effective ecological approach for coral reef restoration [10, 11]. The use of sexual propagation strategies can promote genetic diversity and species evolution, thereby allowing corals to adapt to a changing environment [12–14].

Metamorphisize from planktonic larvae into sedentary polyps represents a key life cycle event for corals. Despite many larvae are releaed by coral parents, only a small percentage of them develop into adult polyps [10]. Stony corals undergo changes in their habitats and morphology during metamorphosis and settlement. As coral larvae grow, they transition from free-swimming, noncalcified larvae into sessile and calcified sedentary polyps [15]. The ability of coral larvae to metamorphose and settlement is crucial to coral development. Environmental degradation complicates coral larvae metamorphosis and settlement [10]. In contrast, crustose coralline algae (CCA) and microbial biofilms have been shown to facilitate these two processes [16–18]. There is increasing recognition that microbes play a role in coral metamorphosis [19–21]. Indeed, studies have shown that bacteria isolated from corals and their surrounding environments, such as *Pseudoalteromonas*, *Marinobacter*, *Cytophaga*, *Thalassomonas*, *Roseivivax*, *Pseudovibrio*, *Acinetobacter*, *Microbulbifer*, and *Metabacillus*, can induce coral larvae metamorphosis and settlement [22–25]. Additionally, it has been reported that tetrabromopyrrole (TBP) from *Pseudoalteromonas* can induce for settlement of multiple Caribbean corals [26, 27]. In Pacific acroporid coral larvae, purified TBP induces metamorphosis without settlement [28]. In addition to TBP, bacteria also produce other factors that can promote coral larval metamorphosis and settelment [22, 25, 29]; however, the underlying mechanisms are unknown.

Free-swimming coral larvae receive settlement cues through sensory neurons, and these cues are then converted into metamorphic signals [30]. A variety of sensory- and signal transduction-related genes, including those coding for ion channels, neuropeptide receptors, and G protein-coupled receptors, have been implicated in coral larval metamorphosis and settlement [28, 30–33]. The metamorphosis and settlement of coral larvae are complex processes that involve the development of coral primary polyps and the establishment of a symbiosis between the coral host and members of the Symbiodiniaceae family of dinoflagellates. Despite the critical role of bacteria in coral larval metamorphosis and settlement, the associated mechanisms remain largely unclear. Given the decline in coral reef ecosystems worldwide, elucidating these mechanisms is crucial for the conservation and restoration of these vital marine habitats. The bacterium *Metabacillus indicus* strain cB07 was previously shown to induce metamorphosis and settlement in larvae of *Pocillopora damicornis* [22], a brooding branching scleractinian coral prevalent in shallow-water habitats across the Indian and Pacific Oceans. Its free-swimming larvae are released with endosymbiotic *Symbiodinium* provided by the parent coral *via* vertical transmission [34–36]. Notably, a single larva of *P. damicornis* was observed to contain approximately 20,000 cells of the Symbiodiniaceae family [37]. In this study, we sought to determine how the transcriptomes of coral hosts and Symbiodiniaceae respond to strain cB07-triggered metamorphosis and settlement of coral larvae and identify the key metabolic pathways involved in these processes. Understanding the molecular mechanisms involved in strain cB07-induced coral settlement opens the possibility of using bacteria as enhancers of settlement in coral reef restoration.

Methods

Coral colony and gamete collection

Twenty adult *P. damicornis* were collected from Luhuitou fringing reefs, Sanya Bay (18°12′ N, 109°28′ E), on August 28, 2019, and immediately transferred to the Tropical Marine Biological Research Station in Hainan. They were placed in individual 20-L containers with flow-through seawater at ambient temperature (28.6±0.2 °C) within a 200-L tank under partially shading (approximately 250 μmol photons m^{-2} s^{-1}). On September 1, 2019, the coral

began brooding, and larval collection was started 2 days later (the brooding peak of *P. damicornis* in Sanya Bay is usually between days 5 and 10 of the lunar calendar). The outflow of each container was passed through a 180-μm cup filter placed at the bottom to capture released larvae. The larvae were collected at 06:30 h and pooled across colonies. On September 6, 2019, the larval collection was completed, and the newly released free-swimming larvae were immediately used for experiments.

Experimental design and sampling

The bacterial cB07 strain was previously isolated from coral tissue and shown to induce metamorphosis and settlement in *Pocillopora damicornis* larvae [22]. Strain cB07 showed 99.93% similarity to *M. indicus* [22]. Two treatment groups were set up, namely, a control group (CK), in which the coral was not inoculated with strain cB07; and a strain cB07 inoculation group (Bac), in which the coral was treated with strain cB07 at a final concentration of 3.2×10^7 cells mL^{-1}. Seawater filtered through 0.2 μm polypropylene filter bags was used for the bacterial addition experiments. Six-well polystyrene culture plates (Corning, Cat No 3536) were used for the determination of coral larval metamorphosis and settlement proportions. Glass Petri dishes (ϕ = 20 cm, height = 3 cm) were used for sampling experiments. All plates and dishes were incubated at 28 ± 1 °C under a 12-h:12-h light/dark cycle (light intensity: 250 μmol photons m^{-2} s^{-1}, provided by an aquarium light). Every 24 h, coral larvae and primary polyps were observed and details were recorded under a dissecting microscope (Nikon SMZ800N, Tokyo, Japan). In the Bac group, larval metamorphosis and settlement were observed at 24 and 48 h post-bacterial addition, respectively. In the CK group, larval metamorphosis and settlement were observed on days 6 and 7 after the commencement of the experiment, respectively. To prevent interference with the larval metamorphosis and settlement processes, the culture water was not replaced throughout the experiments. The metamorphosis and settlement of free-swimming larvae and their subsequent growth into primary polyps are shown in Fig. S1. In this study, metamorphosis refers to flattened, buoyant, non-attached juveniles displaying obvious septal mesenteries radiating from the central mouth region, while settlement refers to larvae that flatten on the surface of the substrate and form primary mesentery filaments (Fig. S1).

After settlement, all free-swimming larvae and the medium were removed. All settled coral primary polyps were incubated in six aquaria each containing 12 L of filtered (0.5 μm) seawater (FSW) and a water pump (AtmanAT-302 with 450 L h^{-1} water flow; Zhongshan, China), forming a 24-h circulating loop. Each aquarium could exchange seawater with a sump containing FSW (0.5 μm). The seawater flow rate was 100 mL min^{-1} for each aquarium, providing a half-fold volume replacement every hour. Twenty primary polyps were randomly selected for labeling. The survival and calcification rate of coral primary polyps were determined after 15 days, with a total of three repeats being performed for each treatment.

Newly released free-swimming and metamorphosed larvae were collected with disposable aseptic straws, while primary polyps were collected with disposable aseptic blades. Additionaly, 100 mL of seawater surrounding each Petri dish was filtered through a 0.22-μm aseptic membrane for the analysis of microbiomes in the cultured water. All samples were frozen in liquid nitrogen and stored at -80 °C for subsequent analysis.

Coral primary polyp survival and asexual reproduction

Daily visual inspections were conducted to monitor the survival rate of coral primary polyps. In cases where the survival status was indeterminate by the naked eye, a dissecting microscope (Nikon SMZ800N, Tokyo, Japan) was utilized.

Budding, indicative of asexual reproduction in corals, results in the formation of new polyps genetically identical to the primary polyp [10]. The budding rate refers to the number of polyps budding as a proportion of the total number of primary polyps. To determine the polyp budding rate and the lateral area, a dissecting microscope (Nikon SMZ800N, Tokyo, Japan) was used to count and photograph the marked larvae every 5 days for 15 days. Four repeats were performed for each group, and 10 coral polyps were selected from each repeat for analysis. The lateral area of larvae was calculated using Photoshop software to determine the horizontal growth area of primary polyps.

Calcification rate

Primary polyps were collected on day 15 and transferred to an aseptic 2-mL centrifuge tube. A total of 10 coral primary polyps from each sample were used to determine calcification rate. Three repetitions were carried out for each treatment. To remove the organic matter in the sample, the primary polyps in the centrifuge tube were immersed in ~0.5 mL of 5% sodium hypochlorite, shaken, mixed well, allowed to stand for 5 min, centrifuged at 4,000 rpm for 1 min, and the supernatant was removed. After adding 0.5 mL of methanol, the primary polyps were shaken and mixed evenly and then centrifuged at 4,000 rpm for 1 min. After removing the supernatant, the precipitate was washed three times with deionized water, allowed to dry naturally, and weighed. The calcification rate of the primary polyps was calculated as their total weight / (the number of weighed larvae × the growth time of the larvae).

Determination of physiological parameters of coral larvae and coral primary polyps

The maximum quantum yields of PSII photochemistry (Fv/Fm) were measured in primary polyps of living coral using a diving-PAM fluorometer equipped with a red-emitting diode (Walz, Germany). One-to-two primary polyps were measured per sample, with three replications.

For the determination of chlorophyll *a* concentrations, coral larvae (60 per sample) and coral primary polyps (20 per sample) were lysed in 1 mL of 90% acetone at 4 °C and shaken three times for 10 s at 1-min intervals on ice. After extraction for 2 h in the dark at 4 °C, the samples were centrifuged for 2 min at 10,000 rpm to remove cellular debris. To quantify the chlorophyll *a* concentration, the absorbance of each sample was measured at 630, 647, and 664 nm using an ELISA plate reader [38]. A 2:1 chloroform/methanol solution was used to extract total lipids from samples (120 coral larvae and 30 coral polyps) five times. The solution was then sonicated (200 W, 1 min), vortexed, and centrifuged at 10,000 rpm for 1 min. The resulting supernatants were transferred to a 10-mL centrifuge tube, washed in 2 mL of 0.88% KCl, centrifuged again at 4,000 rpm for 5 min, and the precipitate was washed three times in a 1:1 methanol/water solution. The extract was dried to constant weight under a stream of pure nitrogen gas at 37 °C [39].

Protein and carbohydrates in the sample were determined by colorimetry at 595 nm and 485 nm, respectively. A 1-mL volume of radioimmunoprecipitation assay (RIPA) buffer (Beyotime, P0013B, Shanghai, China) containing 10 μL of phenylmethanesulfonyl fluoride (100 mM) was added to each sample (60 coral larvae and 20 coral polyps). Slurries were vortexed five times, 5 s each time, and centrifuged for 2 min at 10,000 rpm. The supernatants were subsequently transferred to new 2-mL tubes and stored at −80 °C for further analysis. The supernatants were used for protein extraction using a bicinchoninic acid (BCA) Protein Assay Kit (Beyotime, Shanghai, China) [40] with bovine serum albumin serving as a standard. In a second subsample, carbohydrates were quantified using the phenol-sulfuric acid method with D-glucose serving as a standard [41]. Following dilution with 0.9% NaCl 10 times, the supernatants were vortexed thoroughly. Following the transfer of the diluents (200 μL) into a 10-mL tube, 10 μL of 80% phenol and 1 mL sulfuric acid were added rapidly. After shaking for 10 min, the samples were incubated in a temperature-controlled oven for 30 min, cooled to room temperature, and subjected to an ELISA (absorbance: 485 nm). The chlorophyll *a*, carbohydrate, protein, and lipid concentrations were standardized to the contents of each larva and polyp.

Assessment of strain cB07 through 16 S rRNA gene amplicon sequencing

Total DNA was extracted using the HiPure Soil DNA Kit (Megen, Guangzhou, China) based on the manufacturer's instructions and the concentrations were determined using the Qubit 3.0 Fluorometer High-Sensitivity DNA Kit (Invitrogen, MA, USA). The hypervariable V3 and V4 regions of the bacterial 16 S rRNA gene were amplified using primers 341 forward (5′-CCTACGGGNGGCWGCAG-3′) and 806 reverse (5′-GGACTACHVGGGTATCTAAT-3′). Each PCR run was performed in triplicate. Each 50-μL reaction mixture contained 5 μL of 10× KOD Reaction Buffer, 2 μL of 2 mM dNTPs, 3 μL of 25 mM $MgSO_4$, 1.5 μL of each primer (10 μM), 1 μL of KOD DNA Polymerase (TOYOBO Co., Osaka, Japan), 100 ng of template DNA, and ddH_2O to 50 μL. As a negative control, ddH_2O was used instead of template DNA. The PCR conditions were as follows: 94 °C for 2 min, followed by 30 cycles of 98 °C for 10 s, 62 °C for 30 s, and 68 °C for 30 s, with a final elongation at 68 °C for 5 min. The sizes of the amplicons were assessed by agarose gel electrophoresis. The amplicons were purified with AMPure XP Beads (Beckman Coulter, High Wycombe, UK) according to the manufacturer's instructions and quantified with the ABI StepOnePlus Real-Time PCR System (Life Technologies, Foster City, USA). The purified amplicons were pooled in equimolar amounts and sequenced using the Illumina Hiseq 250 system by Gene Denovo Bio-tech Co. (Guangzhou, China).

Following demultiplexing, the amplicon sequences were merged using FLASH (v.1.2.11) and quality-filtered with FastP (0.19.6) [42, 43]. High-quality sequences were then denoised using the DADA2 [44] plugin within the QIIME 2 (version 2020.2) [45] pipeline, yielding amplicon sequence variants (ASVs). Based on the 16 S rRNA gene sequence from strain cB07, the ASVs obtained from coral fragments were analyzed to confirm the presence and relative abundance of strain cB07 in the coral microbiome.

RNA extraction, library construction, and transcriptome sequencing

Total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA) as instructed by the manufacturer. RNA quality was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). Libraries were prepared using the Hieff NGS Ultima Dual-mode mRNA library Prep Kit (Shanghai Yeasen Biotechnology Co., Ltd, Shanghai, China) and then sequenced on Illumina HiSeq™ 4000 system by Gene Denovo Biotechnology Co. (Guangzhou, China).

Bioinformatics analysis

Reads were filtered by Fastp (version 0.18.0) [43] to obtain high-quality clean reads. Specifically, reads

containing adapters, those with more than 10% unknown nucleotides (N), and those with a *Q*-value ≤ 20 (low-quality reads) was removed. Subsequently, Bowtie2 (version 2.2.8) [46] was used to map reads to ribosome RNA (rRNA) databases, and the rRNA-mapped reads were removed. The remaining clean reads were used for assembly and gene abundance calculation.

An index of the *P. damicornis* reference genome was built, and paired-end clean reads were mapped to this reference genome (Table S1) using HISAT2.2.4 software [47] with the parameter "-rna-strandness RF". The mapped reads of each sample were assembled with StringTie v1.3.1 using a reference-based approach [48, 49]. For each transcription region, a fragment per kilobase of transcript per million mapped reads (FPKM) value was calculated to quantify its expression abundance and variation using RSEM software [50].

As *P. damicornis* forms symbiotic relationships with several species within the Symbiodiniaceae family, a transcriptome analysis of Symbiodiniaceae was conducted using *de novo* assembly. An index of Symbiodiniaceae reference genome (Table S1) was created and the reads that could not be aligned to the reference genome of *P. damicornis* were used for the analysis of the Symbiodiniaceae transcriptome. Symbiodiniaceae transcriptome *de novo* assembly was carried out using the short-read assembling program Trinity [51]. The unigenes obtained by assembly were mapped to the indexed Symbiodiniaceae reference genome using HISAT2.2.4 software [47]. The mapped unigenes were calculated and normalized to RPKM [50].

DESeq2 software was used to analyze differential RNA expression between two groups [52]. Genes with a false discovery rate (FDR) < 0.05 and a fold change ≥ 2 were considered to be differentially expressed. Principal component analysis (PCA) and volcano plots were generated with the R package "gmodels" (http://www.r-project.org/).

A Weighted correlation network analysis (WGCNA) provides evidence that gene expression differences are driven by biological factors rather than stochasticity. Genes that show similar patterns can be clustered and analyzed for their associations with specific traits or phenotypes. In this study, co-expression networks were constructed with the WGCNA package (v1.47) in R [53]. After gene filtering, gene expression values were imported into WGCNA for the construction of co-expression modules using the blockwiseModules automatic network construction function with default settings, except that the power was set to 8 and the min-ModuleSize was set to 50 [54, 55]. Differentially expressed genes (DEGs, with an FDR of < 0.05) due to the inoculation of cB07 were clustered into 17 correlated modules. Eigengenes were used to calculate correlation coefficients with metamorphosis and settlement traits to identify modules that were significantly associated with metamorphosis and settlement. The intramodular connectivity (function soft Connectivity) of each gene was calculated, and genes with a high connectivity tended to be hub genes (q. weighted < 0.01 as a cutoff) [56], that is, those that may act as key regulators of a large subset of genes and work together to perform their biological functions.

Kyoto Encyclopedia of Genes and Genomes (KEGG) was employed for functional enrichment analysis (Bonferroni-corrected *P*-values ≤ 0.05 compared with the whole-transcriptome background) of genes showing differential expression in the presence of strain cB07. KEGG pathway analysis was performed using GOATOOLS (https://github.com/tanghaibao/Goatools) and KOBAS (http://kobas.cbi.pku.edu.cn/home.do).

Statistical analysis

The microbiome and transcriptome bioinformatic analysis was performed in the Omicsmart platform (https://www.omicsmart.com), which provides real-time interactive online data analysis. Statistical analysis of physiological parameters was performed using the Student's *t*-test in SPSS (v.21), with *P*-values < 0.05 considered statistically significant.

Results

Bacteria induced coral larvae metamorphosis and settlement

Statistically significant differences in metamorphosis and settlement proportions were observed between coral larvae inoculated with *M. indicus* strain cB07 (Bac group) and those without inoculation (CK group) (Fig. 1). The Bac group exhibited metamorphosis and settlement proportions of 45% and 70%, respectively, whereas, in the CK group, the respective proportions were only 17% and 20% (Fig. 1).

Physiological parameters of coral larvae and primary polyps

To investigated the effects of strain cB07 on coral larvae and primary polyps, we determined total carbohydrate, total protein, total lipid, and chlorophyll *a* after the concentrations after inoculation (Fig. 2). No significant differences in total carbohydrate, total protein, and chlorophyll *a* contents were detected between newly released larvae and metamorphosed larvae in both the CK and Bac groups (Fig. 2). In the CK group, the total protein content of coral larvae remained stable from the metamorphosis to the settlement phase, while the chlorophyll *a* level notably decreased, accompanied by a significant increase in total carbohydrates and lipids concentrations (Fig. 2). In the Bac group, meanwhile the chlorophyll *a* level also underwent a substantial decline from metamorphosis to

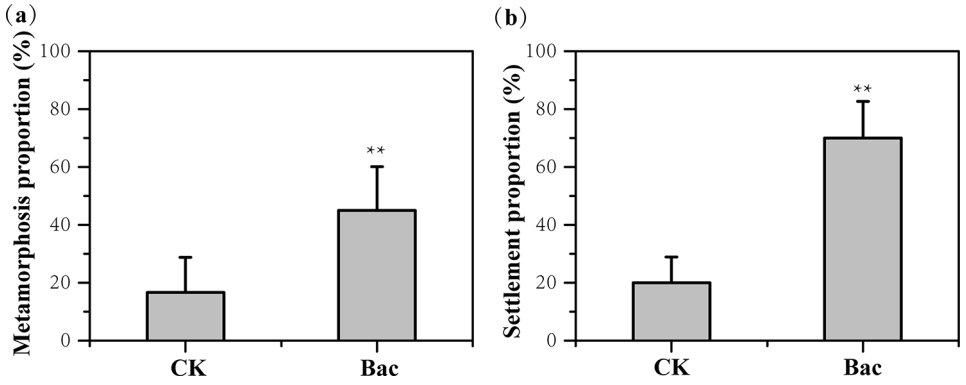

Fig. 1 The metamorphosis and settlement proportion of coral larvae in the control and Bac groups. CK, control group; Bac, bacterial-inoculated group. Error bars represent means ± SE ($n = 6$ replicates, 60 coral larvae per replicate). Asterisks indicate significant differences between the treatment and CK groups based on the Student's *t*- test (**$P < 0.01$)

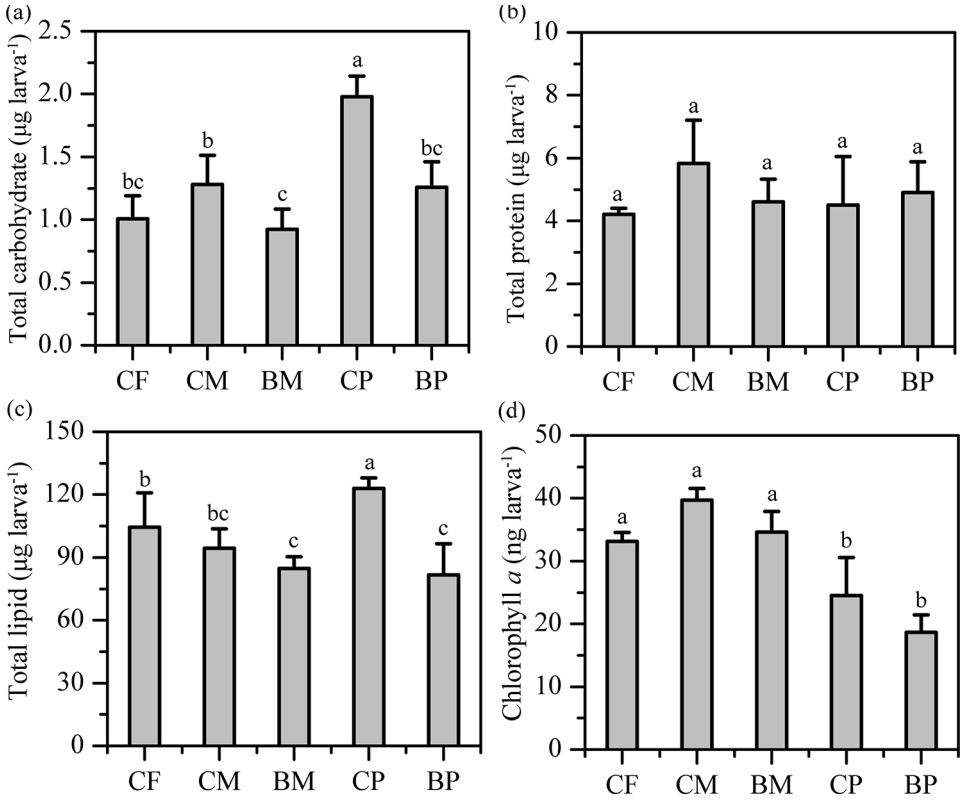

Fig. 2 Comparison of the physiological parameters of coral larvae between the control and treatment groups at different stages. (**a**) total carbohydrate, (**b**) total protein, (**c**) total lipid, and (**d**) chlorophyll *a* content. CF, newly released larvae; CM, Metamorphosis, control (CK) group; BM, Metamorphosis, bacteria inoculation (Bac) group; CP, Settlement, CK group; BP, Settlement, Bac group. Error bars represent means ± SE ($n = 3$ replicates. For total carbohydrate, total protein, and chlorophyll *a*, each replicate consisted of 60 coral larvae; for total lipid, each replicate comprised 120 larvae). Different letters denote significant differences ($P < 0.05$) as determined by the Student's *t*-test

settlement, whereas the total carbohydrate, protein, and lipid levels remained relatively unchanged (Fig. 2). In the Bac group, metamorphosed coral larvae exhibited a significantly reduced carbohydrate content compared with those in the CK group. However, no significant differences in total protein, total lipid, and chlorophyll *a* contents were observed between metamorphosed larvae of the two groups (Fig. 2). Following settlement, total carbohydrate and total lipid levels remained lower in larvae of the Bac group than in those of the CK group. Nevertheless, no significant differences in total protein and chlorophyll *a* contents were observed between settled larvae of the two groups (Fig. 2).

Coral primary polyps were cultured 15 days for the assessment of their total carbohydrates, total protein contents, total lipid contents, chlorophyll *a* contents,

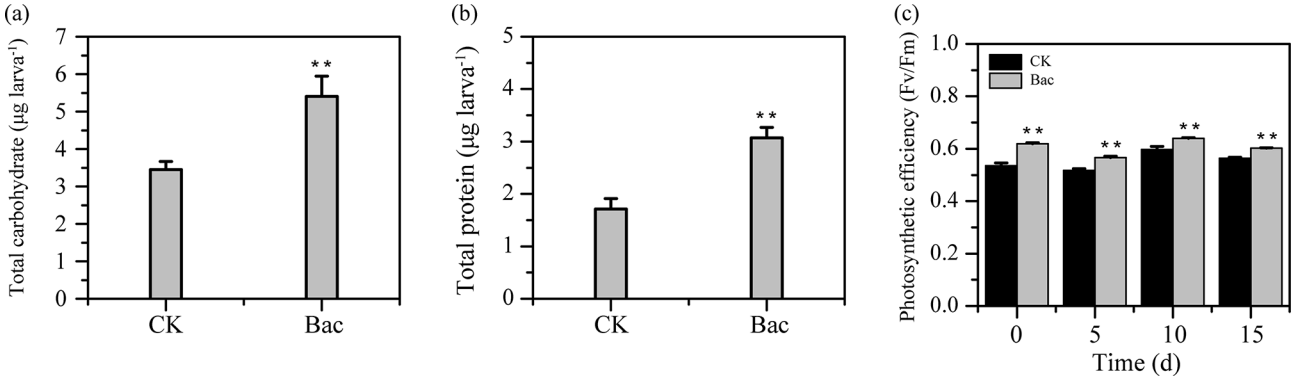

Fig. 3 Physiological profiles of coral primary polyps. (**a**) Total carbohydrate levels, (**b**) total protein levels, and (**c**) photosynthetic efficiency. CK, control group; Bac, bacteria inoculation group. Error bars represent means ± SE ($n = 3$ replicates, 20 primary coral polyps per replicate) (**$P < 0.01$, Student's *t*-test)

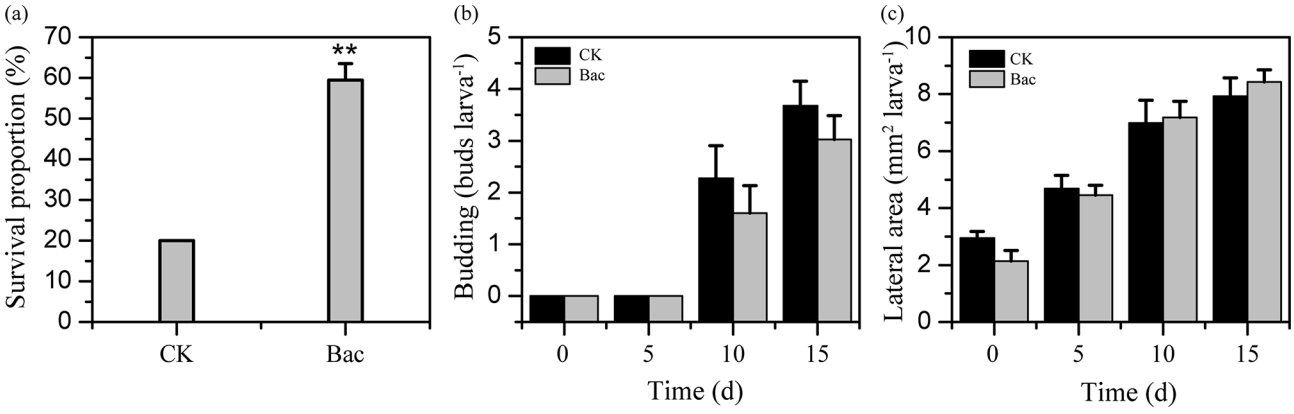

Fig. 4 The survival proportions (**a**), budding rates (**b**), and lateral areas (**c**) of coral primary polyps in the CK and Bac groups. CK, control group; Bac, bacteria inoculation group. Error bars represent means ± SE ($n = 3$ replicates for survival proportion, 20 primary polyps per replicate; $n = 4$ replicates for budding rate and lateral area, 10 coral polys per replicate) (**$P < 0.01$, Student's *t*-test)

and calcification rates, as well as the determination of the photosynthetic efficiency of Symbiodiniaceae. Compared with CK group, the Bac group had significantly higher carbohydrate and protein contents (Fig. 3a, b). In contrast, the total lipid content, the chlorophyll *a* content, and the calcification rate did not differ significantly between the two groups (Fig. S2a-c). The Fv/Fm was measured on days 0, 5, 10, and 15 of culture. At all four sampling time points, the Bac group exhibited significantly higher Fv/Fm values than the CK group (Fig. 3c).

Primary polyp survival and asexual reproduction

In total, 59.5% of the primary polyps survived after 15 days of culture in the Bac group, significantly exceeding ($P < 0.01$) the 20% survival observed in the CK group (Fig. 4a, Fig. S3, $P < 0.01$). However, the budding rates and lateral areas of coral primary polyps were not significantly different between the Bac and CK groups across the four post-settlement time points (Fig. 4b, c, Fig. S4).

Bacterial community variation

A comparative analysis of bacterial communities between the CK and Bac groups revealed that bacterial inoculation exerted a pronounced impact on the community structure of coral larvae at the metamorphosis stage ($P < 0.01$; Table S2). In contrast, no significant variations in the bacterial community structure of larvae were detected between the two groups at the settlement stage ($P > 0.05$; Table S2). Analogous results were obtained when comparing the bacterial community structures in coral culture water for both stages ($P > 0.05$; Table S2). To confirm the presence of the cB07 strain, both Bac and CK samples were analyzed for ASVs during the metamorphosis and settlement phases. In addition to strains added during the experiment, strain cB07 was detected among coral microorganisms and those in the surrounding water in samples of the CK group during both phases (Table S3). Meanwhile, the relative abundance of strain cB07 was significantly higher in the Bac group than that in the CK group both in metamorphosed larvae and primary polyps. Similarly, during the metamorphosis stage, the relative abundance of strain cB07 in the culture water

of strain cB07-inoculated coral larvae was significantly increased compared with that in the CK group. However, at the settlement stage, no significant differences in the relative abundance of strain cB07 were observed between the water samples of the two groups. The inoculated strain cB07 was detected in the surrounding water during the metamorphosis and within primary coral polyps during the settlement stage in the Bac group (Table S3).

Transcriptomics analysis of the coral host

In this study, RNA-seq was used to investigate the differential gene expression linked to cB07-induced metamorphosis and settlement. A total of 709,316,050 reads were generated from total coral larvae and primary polyp samples, yielding 703,180,042 clean, high-quality reads after quality control. Of these clean reads, 329,569,925 were mapped to the coral host genome and were used for the transcriptomics analysis of the coral host (Table S4). PCA was used to visualize global patterns of gene expression and confirmed the separation of groups based on gene expression. In metamorphosed larvae, there was a significant difference in transcript levels between the Bac and CK groups; however, no significant differences in primary polyp transcript levels were found between the two groups (Fig. 5a). The differentially expressed genes (DEGs; FDR < 0.05) resulting from strain cB07 inoculation are displayed in volcano plots in Fig. 5c, d. During the metamorphosis stage, RNA-seq results revealed that

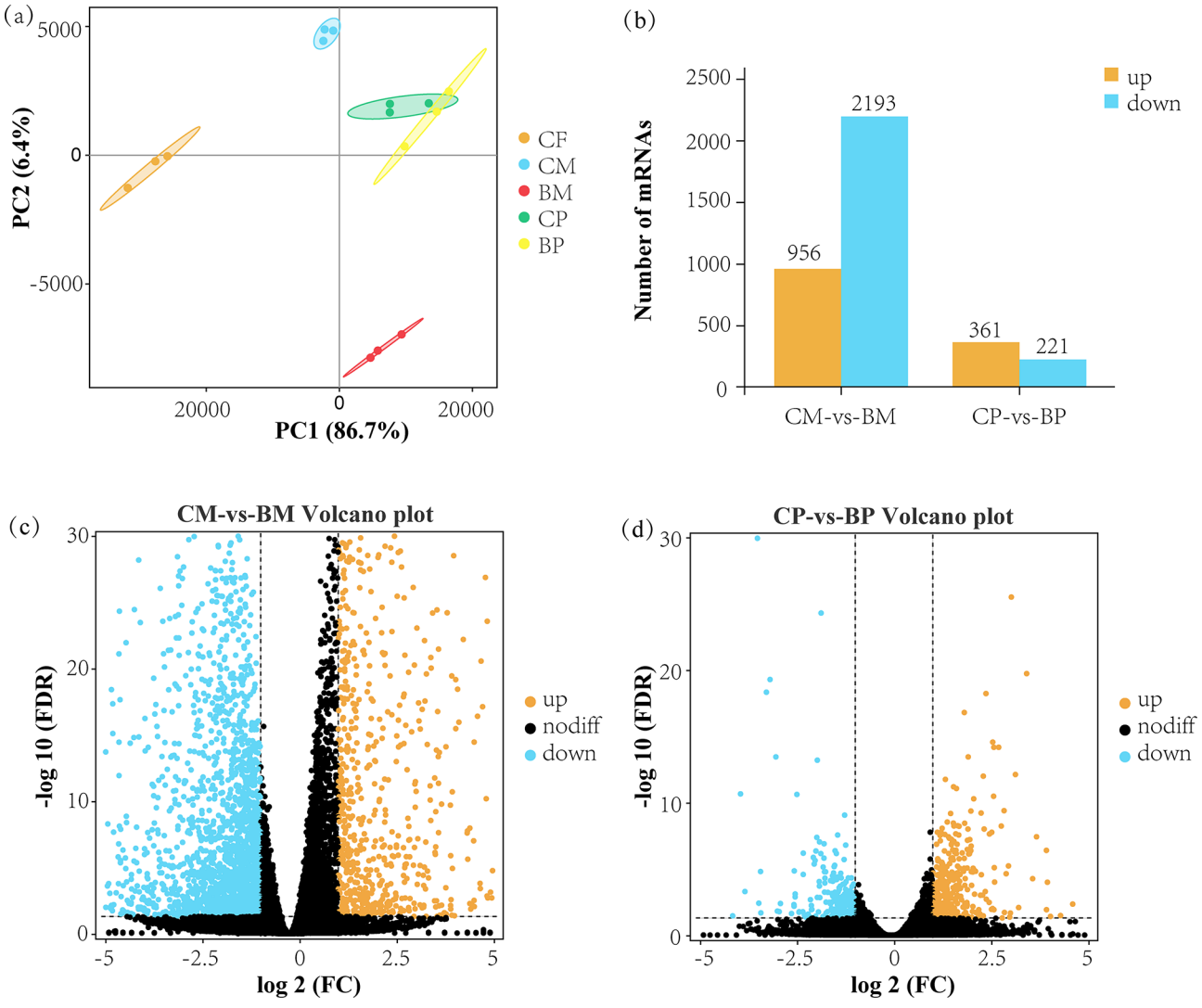

Fig. 5 Differential analysis of mRNA expression levels between the control group and strain cB07 inoculated group. (**a**) Principal component analysis of RNA-seq data. The points represent biological replicates, (**b**) A histogram of differential gene expression among different groups, (**c, d**) Volcano plots showing the relative abundance of transcripts. The transcripts were considered differentially expressed between groups at fold change ≥ 2 and with a false discovery rate < 0.05. The black dots represent genes for which no statistically significant differences were observed. The blue dots represent significantly downregulated genes and the orange dots represent significantly upregulated genes. (CF, Newly released larvae; CM, Metamorphosis, control (CK) group; BM, Metamorphosis, bacteria inoculation (Bac) group; CP, Settlement, CK group; BP, Settlement, Bac group)

3,149 genes were differentially expressed between the CK and Bac groups. Of these genes, 956 exhibited upregulation in the Bac group, while 2,193 genes showed downregulation (Fig. 5b). During the settlement stage, a total of 582 DEGs were identified between the Bac and CK groups, 361 of which were upregulated in the Bac group, while 221 were downregulated (Fig. 5b).

The DEGs (FDR < 0.05) at different stages of coral larvae development were displayed in Venn diagram (Fig. S5). Compared to the CK group, we identified 2,591 DEGs in the Bac group that exhibited significant differential expression as the coral larvae transitioned from the newly released stage to the metamorphosis stage (Fig. S5a). Between the newly released stage and the settlement stage, a subset of 1,869 genes in the Bac group displayed pronounced changes relative to the CK group (Fig. S5b). Furthermore, during the progression from metamorphosis to the settlement stage, 2,696 genes showed differential expression between the two groups (Fig. S5c). The merging of these expression profiles showed that the expression of a total of 5,721 genes was influenced by strain cB07, underscoring the profound impact of this bacterium on the coral larval transcriptome. We next conducted a WGCNA to delve deeper into the associations between the observed DEGs and quantitative traits. The 5,721 DEGs were assigned to 17 coexpression modules and further categorized into three clusters, namely, Clusters A, B, and C (Fig. 6a). Cluster A comprised black (782), blue (842), pink (225), magenta (211), dark red (73), and dark turquoise (53) modules. Within this cluster, the expression patterns of the DEGs showed a trend for upregulation in newly released larvae, contrasting with a trend for downregulation observed during metamorphosis and the settlement phase. Cluster B was composed of six modules, including purple (193), dark green (56), red (423), green-yellow (170), salmon (144), and royal blue (74). The DEG expression patterns in this cluster exhibited a characteristic trajectory, with low levels of expression observed in newly released larvae, subsequently increasing during metamorphosis, and then decreasing in primary polyps. Cluster C encompassed five modules, namely, cyan (134), light yellow (93), green (850), light green (572), and turquoise (826). The DEGs in this cluster displayed minimal expression during the newly released and metamorphosis stages, followed by a marked upregulation in the settlement stage. In summary, these observations confirmed that the three clusters contributed significantly to coral larval development. A module-trait correlation analysis revealed that the metamorphosis proportion was positively correlated with modules within Cluster B, while the settlement proportion was significantly correlated with modules in Cluster

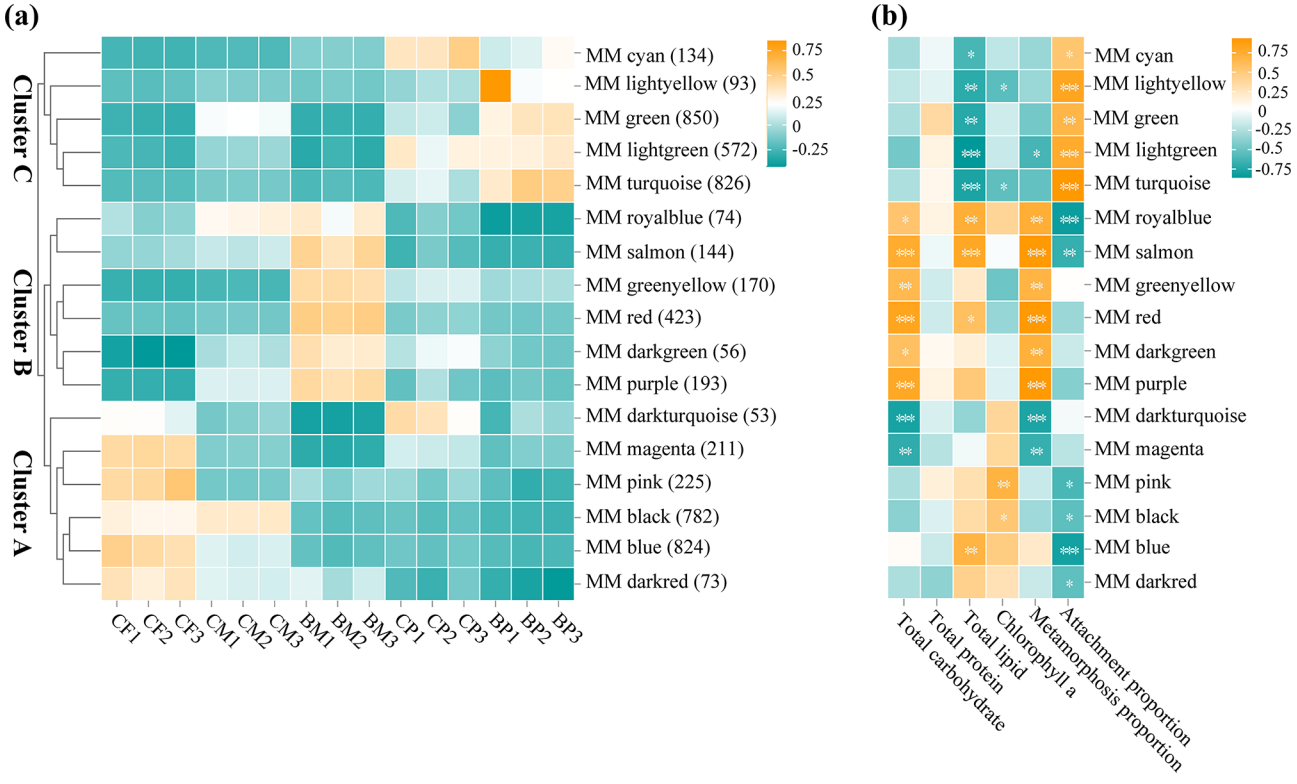

Fig. 6 Hierarchical clustering dendrogram of module eigengens (**a**) and module-trait correlations (**b**). Orange represents a high expression level and blue represents a low expression level; the number of differentially expressed genes in each module is shown in parentheses. MR: metamorphosis proportion, AR: settlement proportion

C (Fig. 6b). Additionally, the dark turquoise and cyan modules demonstrated notable correlations with total carbohydrate and lipid contents, respectively (Fig. 6b).

Genes within Cluster B modules displayed significant enrichment in 11 KEGG pathways (FDR < 0.05), including those associated with genetic information processing, metabolism, organismal systems, and environmental information processing (Table S5). Notable among these pathways were those involved in proteasome; valine, leucine, and isoleucine degradation; protein digestion and absorption; extracellular matrix (ECM)-receptor interaction; fatty acid degradation; porphyrin metabolism; metabolic pathways; fructose and mannose metabolism; carbohydrate absorption and digestion; glyoxylate and dicarboxylate metabolism, and fatty acid metabolism (Fig. 7a). In contrast, genes in Cluster C modules were significantly enriched in 25 KEGG pathways (FDR < 0.05), including those associated with signal transduction, the immune system, and cellular processes (Table S5). Several signaling pathways were identified, including the phospholipase D, Ras, NOD-like receptor, RIG-I, MAPK, TNF, PI3K-Akt, Rap1, and C-type lectin receptor signaling pathways (Fig. 7b).

Transcriptomics analysis of symbiodiniaceae

A total of 373, 610, 117 reads were used for Symbiodiniaceae transcriptomics analysis (Table S4). PCA was subsequently employed to evaluate the transcriptome clustering between groups, revealing that strain cB07 inoculation resulted in minimal variation in gene expression during the coral larval metamorphosis and settlement phases (Fig. 8a). Volcano plots were constructed to delineate the DEGs (FDR < 0.05) in Symbiodiniaceae in response to cB07 inoculation (Fig. 8c, d). A total of 254 and 25 DEGs were detected between the CK and the Bac groups at the metamorphosis and settlement stages, respectively. During metamorphosis, compared with the CK group, 227 Symbiodiniaceae-related genes were upregulated in the Bac group, while 27 were downregulated. When compared with the CK group at the settlement stage, 17 Symbiodiniaceae-related genes were upregulated in the Bac group, and 8 were downregulated (Fig. 8b). In the metamorphosis stage, the upregulated Symbiodiniaceae DEGs were significantly (FDR < 0.05) associated with the Photosynthesis and Peroxisome pathways (Table S6). During the settlement stage, Symbiodiniaceae DEGs were only enriched in the ribosome pathway (FDR < 0.05) (Table S6).

Discussion

The recruitment of coral larvae to benthic marine communities is essential for the sustenance of coral populations. However, this process is intricately influenced by a combination of exogenous and endogenous factors. Using settlement cues as a strategy for engineering coral reef restoration can increase coral reef settlement proportion. Several cues, such as crustose coralline algae (CCA) and reef microbial biofilms [20, 57, 58], are known to promote coral larval metamorphosis and settlement. In the absence of such cues, settlement in many marine invertebrate propagules is delayed or even interrupted. These observations emphasize the importance of these signals for establishing and maintaining marine benthic communities. However, a link between exogenous cues and endogenous factors has yet to be established. Throughout coral larval metamorphosis and settlement, the expression levels of genes involved in general cellular processes, such as macromolecular binding and transport, intracellular and extracellular signal transduction, the regulation of metabolic function, and environmental

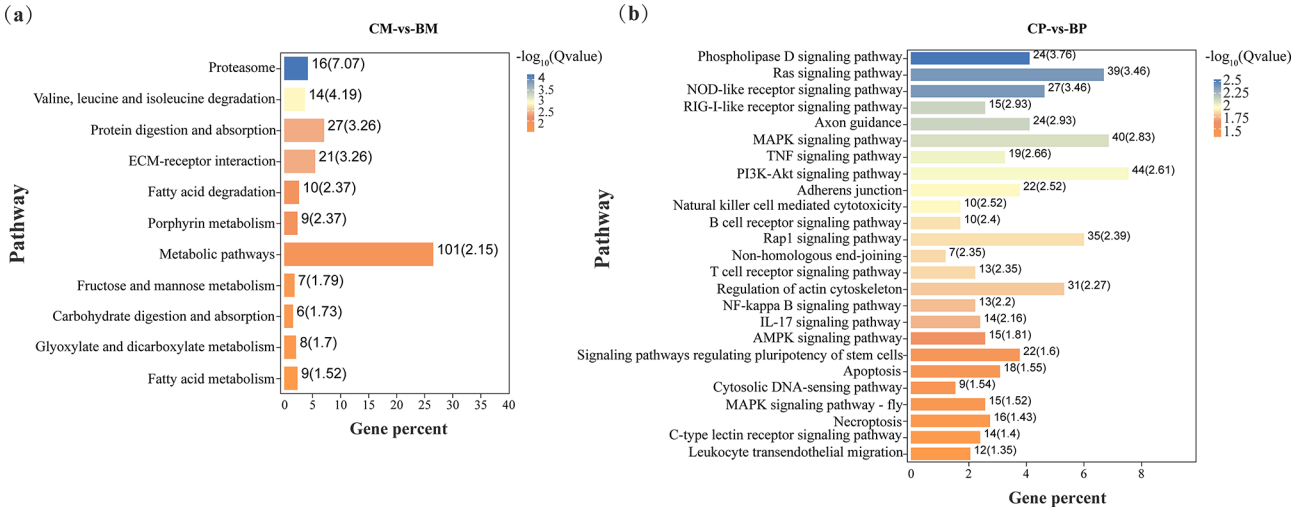

Fig. 7 KEGG pathway enrichment analysis of differentially expressed genes between the control (CK) and bacteria-inoculated coral larvae at the metamorphosis stage (**a**) and the settlement stage (**b**)

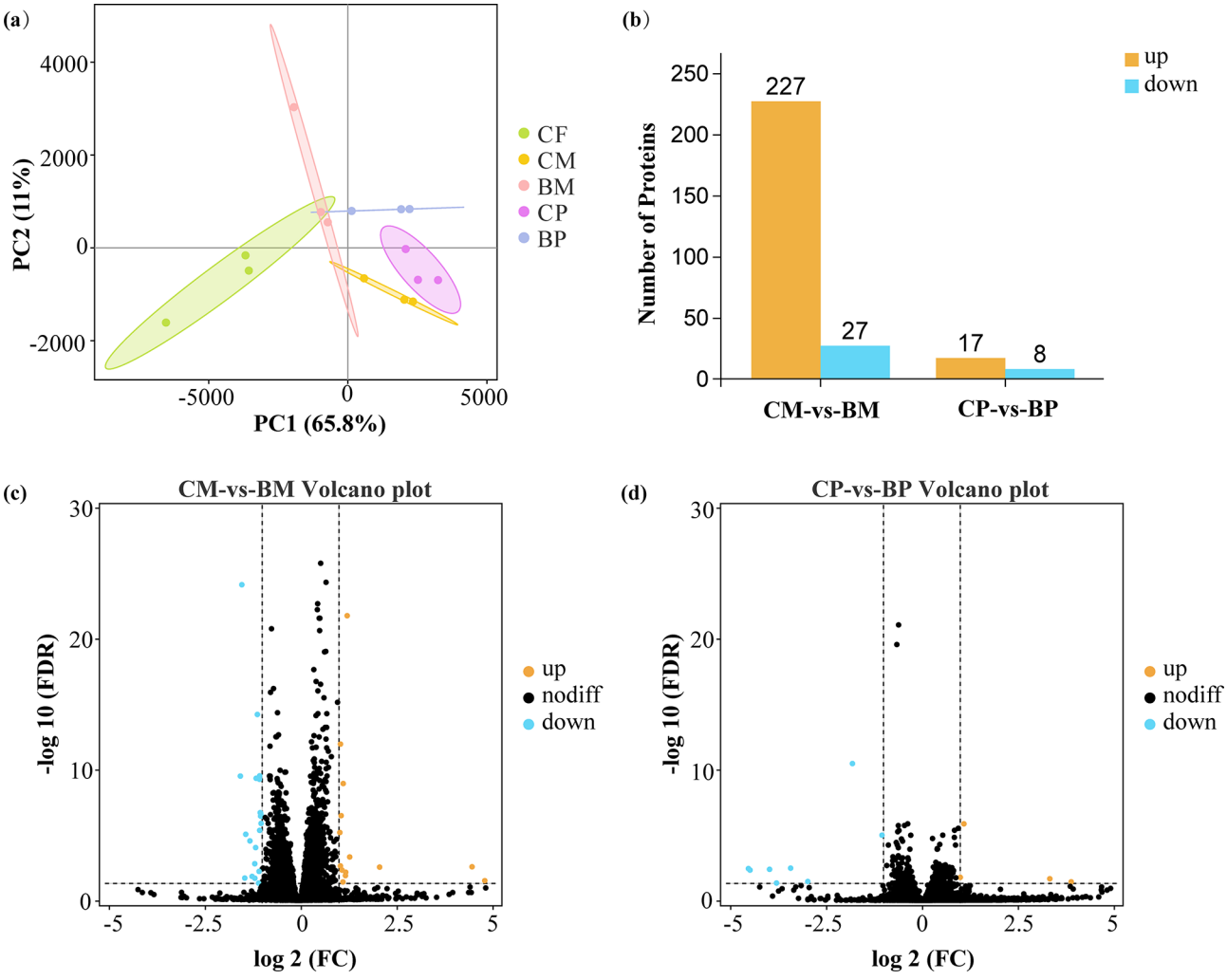

Fig. 8 Differential analysis of the mRNA expression levels between the control group and the strain cB07 inoculation group. (**a**) Principal component analysis of RNA-seq data. The points represent biological replicates. (**b**) A histogram of differential gene expression among different groups. (**c, d**) Volcano plots showing the relative abundance of transcripts. The transcripts were considered differentially expressed between groups at fold change ≥ 2 and with a FDR < 0.05. The black points represent genes for which no statistically significant differences were observed. The blue dots represent significantly down-regulated genes and the orange dots represent significantly upregulated genes. CM, Metamorphosis, control (CK) group; BM, Metamorphosis, bacteria inoculation (Bac) group; CP, Settlement, CK group; BP, Settlement, Bac group

sensing, undergo dynamic changes [15, 30, 31, 33, 57, 59–61]. The expression levels of sensory and signal transduction genes involved in the detection of induction clues, such as those encoding ion channels, neuropeptide signal transduction molecules, and G protein-coupled receptors, are elevated during coral larval development [33]. In corals, different induction clues lead to differences in gene expression [33]. In this study, by integrating transcriptome and phenotypic data analyses, we explored the role of *M. indicus* strain cB07 in inducing coral larval metamorphosis and settlement as well as the subsequent effects on larval development and growth.

The manipulation of beneficial microorganisms for corals (BMCs) has recently been suggested as a promising strategy for promoting resilience and resistance in coral populations [62]. BMCs are known to enhance coral fitness through symbiotic relationships [63, 64]. In this study, the inoculation with strain cB07 led to a significant increase in metamorphosis and settlement proportion in coral larvae. Notably, although strain cB07 was detected among both coral microorganisms and microorganisms in the surrounding water in the CK group, we found that a higher inoculation density was required to effectively induce metamorphosis and settlement in coral larvae relative to that in the Bac group (Fig. S6). The relative high abundance of strain cB07 sequences in inoculated corals indicated the successful establishment of the added strain. Under induction by strain cB07, metamorphosed larvae exhibited significantly reduced total carbohydrate levels compared to control larvae WGCNA

demonstrated that gene modules within Cluster B were significantly positively correlated with the metamorphosis proportion. These modules contained genes that were enriched in metabolic pathways such as amino acid metabolism, protein digestion, fatty acid metabolism, and glucose metabolism. Specifically, genes related to the metabolism of carbohydrates such as fructose, mannose, glyoxylate, and dicarboxylate may account for the observed decrease in carbohydrate content in metamorphosed larvae induced by strain cB07. Furthermore, genes involved in the ECM-receptor interaction pathway were significantly upregulated in metamorphosed larvae of the Bac group. The ECM is known to play a pivotal role in processes such as cell migration, differentiation, tissue morphogenesis, repair, regeneration, and calcium carbonate precipitation in corals [65–67].

Coral primary polyps induced by strain cB07 exhibited significantly reduced total lipid levels compared to controls, potentially due to increased energy expenditure associated with the establishment of a symbiotic relationship and the consequent reduction in buoyancy [68]. This lipid storage loss could alter the displacement of coral larvae through water flow, facilitating the advanced exploration of the settlement substrate. Following the addition of strain cB07, the DEGs in primary polyps were significantly enriched in signal transduction and immune system pathways. Interconnected pathways such as the phosphatidylinositol 3′-kinase (PI3K)-Akt, Rap1, Ras, and MAPK signaling pathways are hypothesized to comprise a meta-signaling pathway integral to cellular differentiation [69]. The PI3K-Akt pathway, in particular, is suggested to mediate the phosphorylation of substrates involved in processes such as apoptosis, protein synthesis, metabolism, and the cell cycle, potentially playing a role in the maintenance and disruption of symbiotic relationships with Symbiodiniaceae [70]. Furthermore, the phospholipase D pathway may be instrumental in the dynamic regulation of Symbiodiniaceae-cnidarian interactions [71].

The immune system, particularly the Nod-like receptor complex, has been implicated in the establishment of functional coral symbiosis [72], possibly by mediating the response to and recognition of algal endosymbionts by coral cells [69]. Additionally, genes associated with C-type lectin receptors (CLRs) were significantly upregulated during coral metamorphosis induced by strain cB07. Lectins are involved in the establishment of endosymbiosis between corals and Symbiodiniaceae, and these genes have previously been shown to be upregulated in coral primary polyps [73]. It has also been reported that genes involved in the CLRs pathway were also significantly upregulated during coral settlement induced by the CCA [59]. The higher abundance of strain cB07 in coral primary polyps suggests that CLR pathway genes may be involved in detecting and acquiring the bacterium from the environment during recruitment, hinting at a link between settlement cues and the initiation of symbiosis.

Coral recruitment is a multifaceted process dependent on both larval settlement and the post-settlement survival and growth of primary polyps [10]. In a 15-day assessment of primary polyp vitality, we observed that strain cB07 did not significantly influence the budding rate, horizontal growth area, or calcification rate, suggesting that the strain exerts a minimal impact on asexual reproduction and calcification in coral polyps. However, polyps inoculated with cB07 demonstrated a significantly higher survival rate than uninoculated ones, along with increased total carbohydrate and protein contents, and enhanced maximum photochemical efficiency. These outcomes indicate that strain cB07 bolsters the photosynthetic capacity of Symbiodiniaceae, thereby facilitating greater energy storage within primary polyps. The heightened photosynthetic efficiency is crucial as Symbiodiniaceae, through photosynthesis, provide a substantial proportion of the host's energy requirements [74]. The establishment of symbiosis between coral larvae and Symbiodiniaceae during larval metamorphosis and settlement involves numerous endosymbiotic genes, including those involved in transport, carbonic anhydrase activity, membrane protein function, metabolic processes, peroxidase activity, apoptosis, and inflammation [60, 75–77]. Additionally, miRNA of endosymbiotic algae was reported to be highly expressed during the early stages of endosymbiosis, suggesting that it plays a role in the regulation of the symbiotic relationship [60]. The brooded larvae of *P. damicornis*, provisioned with Symbiodiniaceae from the maternal colony, displayed minimal changes in Symbiodiniaceae gene expression during coral settlement induced by strain cB07. Despite the subtlety of the changes, the detected DEGs were significantly enriched in the photosynthesis and peroxisome pathways during coral larval metamorphosis, with a notable upregulation of photosynthesis-related genes. This upregulation indicates that inoculation with strain cB07 enhances photosynthetic efficiency, aligning with the recorded increase in photosynthetic efficiency values. This enhanced photosynthesis may increase nutrient provision to coral primary polyps, thereby augmenting their energy levels. During coral metamorphosis and settlement induced by CCA, many genes associated with peroxisomes were also reported to be upregulated [59]. This suggests that peroxisome-related genes must be upregulated to neutralize the oxidative stress potentially caused by an overabundance of reactive oxygen species. During the settlement process, the DEGs were significantly enriched in ribosomal pathways, indicative of an increase in protein synthesis, and further hinting at a potential

mechanism by which strain cB07 may stimulate Symbiodiniaceae growth rates. Furthermore, the upregulation of endocytosis pathway-related genes in Symbiodiniaceae indicates that these genes play a beneficial role in resuming and maintaining the symbiotic relationship between corals and their algal partners [60].

Conclusions

The establishment of strain cB07 in coral larvae induced their metamorphosis and settlement, leading to the generation of post-settlement polyps that exhibited enhanced survival rates and improved health status. During this process, the genes and pathways pivotal to the establishment of a symbiotic relationship between the coral larvae and the bacteria were significantly upregulated. In contrast to the coral larvae, the Symbiodiniaceae endosymbionts were minimally impacted by the strain cB07-induced metamorphosis and settlement. However, the upregulation of photosynthesis-related genes in the Symbiodiniaceae suggests that inoculation with strain cB07 promoted coral polyp photosynthesis, which is advantageous for the health and vitality of the coral holobiont. The capacity of strain cB07 to effectively induce coral larval metamorphosis and settlement, coupled with its role in enhancing the symbiotic relationship, renders it a promising candidate for applications in coral reef restoration efforts.

Abbreviations

ASV	Amplicon Sequence Variants
BMC	Beneficial Microorganisms for Corals
CCA	Crustose Coralline Algae
CLR	C-Type Lectin Receptors
DEG	Differentially expressed genes
FDR	False Discovery Rate
PCA	Principal Component Analysis
CK	Control Group
Bac	Strain cB07 Inoculation Group

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-024-10742-z.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Acknowledgements

This research was supported by the National Key Research and Development Program of China (2022YFC3103602; 2022YFC3102004; 2022YFC3102003), the National Natural Science Foundation of China (No. 41976147), the NSFC-Shandong Joint Fund (No. U2106208), and the Science and Technology Planning Project of Guangdong Province of China (2020B1212060058). We are grateful to Guangzhou Genedenovo Biotechnology Co., Ltd for assisting in sequencing analysis.

Author contributions

YZ and JD conceived and designed the study. YZ, XT, XG and HW acquired and analyzed the data. YZ and XT drew graphs. YZ drafted the manuscript. YZ, QY, HS, and JL reviewed the manuscript and provided valuable edits. All authors have read and approved the submitted version of the manuscript.

Data availability

Raw reads generated in this work were deposited in the NCBI Sequence Read Archive under the BioProject accession number PRJNA931661.

Declarations

Ethics approval and consent to participate

Coral sampling and experiment were conducted in accordance with the relevant guidelines and regulations, and were approved by the Department of Agriculture and Rural Areas of Hainan Provinces.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Ocean School, Yantai University, Yantai 264005, China
[2]CAS Key Laboratory of Tropical Marine Bio-Resources and Ecology, Guangdong Provincial Key Laboratory of Applied Marine Biology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China
[3]Sanya National Marine Ecosystem Research Station, Tropical Marine Biological Research Station in Hainan, Chinese Academy of Sciences, Sanya 572000, China
[4]Guangdong Provincial Observation and Research Station for Coastal Upwelling Ecosystem, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Shantou 515041, PR China

Received: 19 October 2023 / Accepted: 28 August 2024
Published online: 06 September 2024

References

1. Crossland CJ, Hatcher BG, Smith SV. Role of coral reefs in global ocean production. Coral Reefs. 1991;10(2):55–64.
2. Moberg F, Folke C. Ecological goods and services of coral reef ecosystems. Ecol Econ. 1999;29(2):215–33.
3. De'ath G, Fabricius KE, Sweatman H, Puotinen M. The 27-year decline of coral cover on the great barrier reef and its causes. Proc Natl Acad Sci U S A. 2012;109(44):17995–9.
4. Duprey NN, Yasuhara M, Baker DM. Reefs of tomorrow: eutrophication reduces coral biodiversity in an urbanized seascape. Glob Chang Biol. 2016;22(11):3550–65.
5. Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, et al. Coral reefs under rapid climate change and ocean acidification. Science. 2007;318(5857):1737–42.
6. Hughes TP, Anderson KD, Connolly SR, Heron SF, Kerry JT, Lough JM, Baird AH, Baum JK, Berumen ML, Bridge TC, et al. Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. Science. 2018;359(6371):80–3.
7. Gilmour JP, Smith LD, Heyward AJ, Baird AH, Pratchett MS. Recovery of an isolated coral reef system following severe disturbance. Science. 2013;340(6128):69–71.
8. Epstein N, Bak RPM, Rinkevich B. Strategies for Gardening Denuded Coral reef areas: the Applicability of using different types of Coral Material for reef restoration. Restor Ecol. 2001;9(4):432–42.
9. Lirman D, Schopmeyer S. Ecological solutions to reef degradation: optimizing coral reef restoration in the Caribbean and Western Atlantic. PeerJ. 2016;4:e2597.
10. Randall CJ, Negri AP, Quigley KM, Foster T, Ricardo GF, Webster NS, Bay LK, Harrison PL, Babcock RC, Heyward AJ. Sexual production of corals for reef restoration in the Anthropocene. Mar Ecol Prog Ser. 2020;635:203–32.

11. Doropoulos C, Elzinga J, ter Hofstede R, van Koningsveld M, Babcock RC. Optimizing industrial-scale coral reef restoration: comparing harvesting wild coral spawn slicks and transplanting gravid adult colonies. Restor Ecol. 2019;27(4):758–67.
12. van Oppen MJH, Gates RD, Blackall LL, Cantin N, Chakravarti LJ, Chan WY, Cormick C, Crean A, Damjanovic K, Epstein H, et al. Shifting paradigms in restoration of the world's coral reefs. Glob Chang Biol. 2017;23(9):3437–48.
13. van Oppen MJ, Oliver JK, Putnam HM, Gates RD. Building coral reef resilience through assisted evolution. Proc Natl Acad Sci U S A. 2015;112(8):2307–13.
14. Henry JA, O'Neil KL, Pilnick AR, Patterson JT. Strategies for integrating sexually propagated corals into Caribbean reef restoration: experimental results and considerations. Coral Reefs. 2021;40(5):1667–77.
15. Grasso LC, Negri AP, Foret S, Saint R, Hayward DC, Miller DJ, Ball EE. The biology of coral metamorphosis: molecular responses of larvae to inducers of settlement and metamorphosis. Dev Biol. 2011;353(2):411–9.
16. Webster NS, Smith LD, Heyward AJ, Watts JE, Webb RI, Blackall LL, Negri AP. Metamorphosis of a scleractinian coral in response to microbial biofilms. Appl Environ Microbiol. 2004;70(2):1213–21.
17. Heyward AJ, Negri AP. Natural inducers for coral larval metamorphosis. Coral Reefs. 1999;18(3):273–9.
18. Harrington L, Fabricius K, De'ath G, Negri A. Recognition and Selection of Settlement Substrata Determine Post-settlement Survival in corals. Ecology. 2004;85(12):3428–37.
19. Shikuma NJ, Antoshechkin I, Medeiros JM, Pilhofer M, Newman DK. Stepwise metamorphosis of the tubeworm Hydroides elegans is mediated by a bacterial inducer and MAPK signaling. Proc Natl Acad Sci U S A. 2016;113(36):10097–102.
20. Siboni N, Abrego D, Puill-Stephan E, King WL, Bourne DG, Raina J-B, Seymour JR, Harder T. Crustose coralline algae that promote coral larval settlement harbor distinct surface bacterial communities. Coral Reefs. 2020;39(6):1703–13.
21. Negri AP, Webster NS, Hill RT, Heyward AJ. Metamorphosis of broadcast spawning corals in response to bacteria isolated from crustose algae. Mar Ecol Prog Ser. 2001;223:121–31.
22. Zhang Y, Zhang Y, Yang Q, Ling J, Tang X, Zhang W, Dong J. Complete genome sequence of metabacillus sp. cB07, a bacterium inducing settlement and metamorphosis of coral larvae. Mar Genomics. 2021;60:100877.
23. Petersen L-E, Moeller M, Versluis D, Nietzer S, Kellermann MY, Schupp PJ. Mono- and multispecies biofilms from a crustose coralline alga induce settlement in the scleractinian coral Leptastrea purpurea. Coral Reefs. 2021;40(2):381–94.
24. Tran C, Hadfield MG. Larvae of Pocillopora damicornis (Anthozoa) settle and metamorphose in response to surface-biofilm bacteria. Mar Ecol Prog Ser. 2011;433:85–96.
25. Sharp KH, Sneed JM, Ritchie KB, McDaniel L, Paul VJ. Induction of larval settlement in the reef Coral Porites astreoides by a cultivated Marine Roseobacter Strain. Biol Bull. 2015;228(2):98–107.
26. Sneed JM, Sharp KH, Ritchie KB, Paul VJ. The chemical cue tetrabromopyrrole from a biofilm bacterium induces settlement of multiple Caribbean corals. Proc Biol Sci 2014, 281(1786).
27. Tebben J, Tapiolas DM, Motti CA, Abrego D, Negri AP, Blackall LL, Steinberg PD, Harder T. Induction of larval metamorphosis of the coral Acropora millepora by tetrabromopyrrole isolated from a Pseudoalteromonas bacterium. PLoS ONE. 2011;6(4):e19082.
28. Siboni N, Abrego D, Seneca F, Motti CA, Andreakis N, Tebben J, Blackall LL, Harder T. Using bacterial extract along with differential gene expression in Acropora millepora larvae to decouple the processes of attachment and metamorphosis. PLoS ONE. 2012;7(5):e37774.
29. Jorissen H, Galand PE, Bonnard I, Meiling S, Raviglione D, Meistertzheim AL, Hedouin L, Banaigs B, Payri CE, Nugues MM. Coral larval settlement preferences linked to crustose coralline algae with distinct chemical and microbial signatures. Sci Rep. 2021;11(1):14610.
30. Hayward DC, Hetherington S, Behm CA, Grasso LC, Foret S, Miller DJ, Ball EE. Differential gene expression at coral settlement and metamorphosis–a subtractive hybridization study. PLoS ONE. 2011;6(10):e26411.
31. Strader ME, Aglyamova GV, Matz MV. Molecular characterization of larval development from fertilization to metamorphosis in a reef-building coral. BMC Genomics. 2018;19(1):17.
32. Attenborough RMF, Hayward DC, Wiedemann U, Foret S, Miller DJ, Ball EE. Expression of the neuropeptides RFamide and LWamide during development of the coral Acropora millepora in relation to settlement and metamorphosis. Dev Biol. 2019;446(1):56–67.
33. Ishii Y, Hatta M, Deguchi R, Kawata M, Maruyama S. Gene expression alterations from reversible to irreversible stages during coral metamorphosis. Zoological Lett. 2022;8(1):4.
34. Harrison PL, Wallace CC. Reproduction, dispersal and recruitment of scleractinian corals. Coral reefs. Volume 25. Elsevier; 1990. pp. 133–207.
35. Rivest EB, Hofmann GE. Responses of the metabolism of the Larvae of Pocillopora damicornis to Ocean Acidification and warming. PLoS ONE. 2014;9(4):e96172.
36. Jiang L, Zhang F, Guo M, Guo Y, Zhang Y, Zhou G, Cai L, Lian J, Qian P, Huang H. Increased temperature mitigates the effects of ocean acidification on the calcification of juvenile Pocillopora damicornis, but at a cost. Coral Reefs. 2018;37:71–9.
37. Jiang L. Physiological responses of early stages of the reef coral *Pocillopora damicornis* to ocean warming and acidification. University of Chinese Academy of Sciences; 2018.
38. Jeffrey SW, Humphrey GF. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochemie Und Physiologie Der Pflanzen. 1975;167(2):191–4.
39. Harii S, Yamamoto M, Hoegh-Guldberg O. The relative contribution of dinoflagellate photosynthesis and stored lipids to the survivorship of symbiotic larvae of the reef-building corals. Mar Biol. 2010;157(6):1215–24.
40. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72(1–2):248–54.
41. DuBois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric Method for Determination of Sugars and related substances. Anal Chem. 2002;28(3):350–6.
42. Magoc T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics. 2011;27(21):2957–63.
43. Chen S, Zhou Y, Chen Y, Gu J. Fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics. 2018;34(17):i884–90.
44. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13(7):581–3.
45. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019;37(8):852–7.
46. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012;9(4):357–9.
47. Kim D, Langmead B, Salzberg SL. HISAT: a fast spliced aligner with low memory requirements. Nat Methods. 2015;12(4):357–60.
48. Pertea M, Pertea GM, Antonescu CM, Chang TC, Mendell JT, Salzberg SL. StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. Nat Biotechnol. 2015;33(3):290–5.
49. Pertea M, Kim D, Pertea GM, Leek JT, Salzberg SL. Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. Nat Protoc. 2016;11(9):1650–67.
50. Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics. 2011;12:323.
51. Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, et al. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol. 2011;29(7):644–52.
52. Love MI, Huber W, Anders S. Moderated estimation of Fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15(12):550.
53. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics. 2008;9(1):559.
54. Moschen S, Higgins J, Di Rienzo JA, Heinz RA, Paniego N, Fernandez P. Network and biosignature analysis for the integration of transcriptomic and metabolomic data to characterize leaf senescence process in sunflower. BMC Bioinformatics. 2016;17(Suppl 5):174.
55. Botia JA, Vandrovcova J, Forabosco P, Guelfi S, D'Sa K, United Kingdom Brain Expression C, Hardy J, Lewis CM, Ryten M, Weale ME. An additional k-means clustering step improves the biological features of WGCNA gene co-expression networks. BMC Syst Biol. 2017;11(1):47.
56. Xu P, Yang J, Liu J, Yang X, Liao J, Yuan F, Xu Y, Liu B, Chen Q. Identification of glioblastoma gene prognosis modules based on weighted gene co-expression network analysis. BMC Med Genomics. 2018;11(1):96.
57. Freckelton ML, Nedved BT, Hadfield MG. Induction of invertebrate larval settlement; different Bacteria, different mechanisms? Sci Rep. 2017;7:42557.

58. Gomez-Lemos LA, Doropoulos C, Bayraktarov E, Diaz-Pulido G. Coralline algal metabolites induce settlement and mediate the inductive effect of epiphytic microbes on coral larvae. Sci Rep. 2018;8(1):17557.
59. Meyer E, Aglyamova GV, Matz MV. Profiling gene expression responses of coral larvae (Acropora millepora) to elevated temperature and settlement inducers using a novel RNA-Seq procedure. Mol Ecol. 2011;20(17):3599–616.
60. Yuyama I, Ishikawa M, Nozawa M, Yoshida MA, Ikeo K. Transcriptomic changes with increasing algal symbiont reveal the detailed process underlying establishment of coral-algal symbiosis. Sci Rep. 2018;8(1):16802.
61. Deboer ML, Krupp DA, Weis VM. Proteomic and transcriptional analyses of coral larvae newly engaged in symbiosis with dinoflagellates. Comp Biochem Physiol Part D Genomics Proteom. 2007;2(1):63–73.
62. Peixoto RS, Rosado PM, Leite DC, Rosado AS, Bourne DG. Beneficial Microorganisms for Corals (BMC): proposed mechanisms for Coral Health and Resilience. Front Microbiol. 2017;8:341.
63. Santoro EP, Borges RM, Espinoza JL, Freire M, Messias C, Villela HDM, Pereira LM, Vilela CLS, Rosado JG, Cardoso PM et al. Coral microbiome manipulation elicits metabolic and genetic restructuring to mitigate heat stress and evade mortality. Sci Adv 2021, 7(33).
64. Damjanovic K, van Oppen MJH, Menendez P, Blackall LL. Experimental inoculation of Coral recruits with marine Bacteria indicates scope for Microbiome Manipulation in Acropora tenuis and Platygyra daedalea. Front Microbiol. 2019;10:1702.
65. Tucker RP, Adams JC. Adhesion networks of cnidarians: a postgenomic view. Int Rev Cell Mol Biol. 2014;308:323–77.
66. Mass T, Drake JL, Haramaty L, Rosenthal Y, Schofield OM, Sherrell RM, Falkowski PG. Aragonite precipitation by proto-polyps in coral cell cultures. PLoS ONE. 2012;7(4):e35049.
67. Helman Y, Natale F, Sherrell RM, Lavigne M, Starovoytov V, Gorbunov MY, Falkowski PG. Extracellular matrix production and calcium carbonate precipitation by coral cells in vitro. Proc Natl Acad Sci U S A. 2008;105(1):54–8.
68. Szmant A, Meadows MG. Developmental changes in coral larval buoyancy and vertical swimming behavior: implications for dispersal and connectivity. Proc 10th Int Coral Reef Symp. 2006;1:431–7.
69. Shumaker A, Putnam HM, Qiu H, Price DC, Zelzion E, Harel A, Wagner NE, Gates RD, Yoon HS, Bhattacharya D. Genome analysis of the rice coral Montipora capitata. Sci Rep. 2019;9(1):2571.
70. Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat Rev Genet. 2006;7(8):606–19.
71. Simona F, Zhang H, Voolstra CR. Evidence for a role of protein phosphorylation in the maintenance of the cnidarian-algal symbiosis. Mol Ecol. 2019;28(24):5373–86.
72. Hamada M, Shoguchi E, Shinzato C, Kawashima T, Miller DJ, Satoh N. The complex NOD-like receptor repertoire of the coral Acropora digitifera includes novel domain combinations. Mol Biol Evol. 2013;30(1):167–76.
73. Grasso LC, Maindonald J, Rudd S, Hayward DC, Saint R, Miller DJ, Ball EE. Microarray analysis identifies candidate genes for key roles in coral development. BMC Genomics. 2008;9:540.
74. Yellowlees D, Rees TA, Leggat W. Metabolic interactions between algal symbionts and invertebrate hosts. Plant Cell Environ. 2008;31(5):679–94.
75. Yuyama I, Watanabe T, Takei Y. Profiling differential gene expression of symbiotic and aposymbiotic corals using a high coverage gene expression profiling (HiCEP) analysis. Mar Biotechnol (NY). 2011;13(1):32–40.
76. Voolstra CR, Schwarz JA, Schnetzer J, Sunagawa S, Desalvo MK, Szmant AM, Coffroth MA, Medina M. The host transcriptome remains unaltered during the establishment of coral-algal symbioses. Mol Ecol. 2009;18(9):1823–33.
77. Lehnert EM, Mouchka ME, Burriesci MS, Gallo ND, Schwarz JA, Pringle JR. Extensive differences in gene expression between symbiotic and aposymbiotic cnidarians. G3 (Bethesda). 2014;4(2):277–95.

Publisher's note