Probiotics reshape the coral microbiome in situ without affecting the surrounding environment

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Article

Keywords:

Posted Date: October 31st, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3414265/v1

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Additional Declarations: There is NO Competing Interest.

Version of Record: A version of this preprint was published at Communications Biology on April 9th, 2024. See the published version at https://doi.org/10.1038/s42003-024-06135-3.
Abstract

Beneficial Microorganisms for Corals (BMCs), or probiotics, enhance coral resilience against stressors in laboratory trials, being the only sustainable treatment currently explored to retain threatened native corals. However, the ability of probiotics to restructure the coral microbiome in situ is yet to be determined. To elucidate this, we inoculated BMCs on Pocillopora verrucosa colonies in situ in the Red Sea for three months. BMCs significantly influenced the coral microbiome, while those of the surrounding seawater and sediment appeared unchanged. The inoculated genera Halomonas and Pseudoalteromonas were significantly enriched in probiotic-treated corals. Furthermore, probiotic treatment correlated with an increase in beneficial groups (e.g., Ruegeria and Limosilactobacillus), while potential coral pathogens, like Vibrio, decreased in abundance. Notably, treated and non-treated corals remained healthy throughout the experiment. Our data indicate the feasibility of using probiotics in real-world conservation efforts through beneficial restructuring of the coral microbiome without off-target changes in the surrounding environment.

Main

Coral reefs are important ecosystems in the marine environment, supporting a wide range of organisms and providing essential ecosystem services to human populations. Despite their ecological and economic importance, it is estimated that by 2030 approximately 60% of coral reefs worldwide will be under threat due to global (e.g. ocean warming due to increased CO₂ emissions) and local impacts (e.g. water pollution, coastal development, and overfishing). While mitigating ocean warming and local impacts is crucial for preserving coral reefs, there has been a growing focus on active intervention strategies aimed at enhancing coral natural resilience to cope with the already established negative effects of environmental stressors. One strategy is to use Beneficial Microorganisms for Corals (BMCs, or coral probiotics) to rehabilitate the coral microbiome, promoting coral health. In aquarium experiments, probiotics have shown beneficial effects in promoting coral growth and mitigating the effects of pollution, bleaching, and disease, even preventing coral mortality. This approach involves isolating coral-associated bacteria with subsequent screening for beneficial traits for the coral holobiont and developing customized probiotic cocktails for corals.

The “coral probiotic hypothesis” suggested that microbial communities in the coral mucus and tissue layers act as a defense against pathogens and assist in rapid adaptation to changing environmental conditions. Later studies provided further evidence of the beneficial interactions between a diverse community of coral-associated bacteria and the coral holobiont, through mechanisms such as nutrient cycling, production of antimicrobial substances, degradation of sulfur substances and toxic compounds, scavenging reactive oxygen species, and facilitation of coral larval settlement. These and additional putative mechanisms were included within a framework proposing the active use of these beneficial traits as probiotics.
Since the proof of concept showing that BMCs can improve coral health and mitigate damage caused by oil spills, pathogen infection, and thermal bleaching\textsuperscript{12,13}, further research has explored the underlying mechanisms, physiological effects, and application of BMCs across different coral species and life stages (e.g.\textsuperscript{14–18,34–37}). Recent advancements include an enhanced understanding of BMC mechanisms, selection of probiotic strains based on genomic screening, and the exploration of other microbial therapies and bacterial experimental evolution\textsuperscript{19,38–41}.

Despite these promising developments, the ability to restructure the coral microbiome using probiotics \textit{in situ} was not previously tested, and the overall effect of BMCs administration on the coral-associated microbiome and surrounding reef ecosystem remains unclear\textsuperscript{19}. In this study, we conducted repeated \textit{in situ} probiotic inoculations over a three-month period on colonies of the scleractinian \textit{Pocillopora verrucosa} (Ellis and Solander, 1786) to investigate changes in their microbiome, surrounding microbial communities and coral health. Three of the inoculated BMC genera were enriched in the coral microbiome, which was aligned with an overall coral’s microbiome restructuring. Importantly, the probiotic application did not affect the bacterial communities in the surrounding seawater and sediment. This study provides the first experimental evidence of the feasibility of restructuring the coral microbiome using probiotics in the ocean without affecting the microbial communities of the surrounding environment.

\textbf{Results}

\textbf{Probiotic consortium selection and assemblage}

The probiotic consortium was composed of six bacterial strains isolated from visually healthy colonies of \textit{Pocillopora verrucosa} (two \textit{Pseudoalteromonas galatheae} and two \textit{Cobetia amphilecti}), \textit{Stylophora pistillata} (Esper, 1792) (one \textit{Halomonas} sp.), and \textit{Galaxea fascicularis} (Linnaeus, 1767) (one \textit{Suctliella} sp.) collected in the central Red Sea. The BMC strains were selected based upon their potentially beneficial traits via \textit{in vitro} testing (Table S1). We conducted a three-month probiotic inoculation (three times per week) \textit{in situ} on visually healthy colonies of \textit{P. verrucosa} in the central Red Sea (T1-T3). We monitored the bacterial community of the coral before (T1), during (T2), and at the end of the probiotic inoculations (T3), as well as five months after the last inoculation (T4), covering seasonal variations (represented here as seasonal seawater temperature changes in the study site). We also assessed changes in nearby water and sediment bacterial communities before and after the last inoculations (T1 and T3) through 16S ribosomal RNA (rRNA) gene amplicon sequencing. Additionally, we monitored the corals’ physiology by measuring the photosynthetic efficiency of the Symbiodiniaceae present within the coral tissue \textit{in situ} (T1-T4) and the corals’ heat response in CBASS controlled experiments (T1-T4) (Fig. 1A-D) (see details in methods).

\textit{Effect of the probiotic in situ inoculation on the coral microbiome and surrounding environment}
We assessed the effect of coral probiotics (BMCs) on the microbiome of visually healthy *P. verrucosa* colonies by monitoring the bacterial community at four sampling points (T1, T2, T3 and T4) interspersed between inoculations. Amplicon 16S rRNA gene sequencing revealed that the long-term probiotic inoculation *in situ* significantly changed the coral microbiome of the microbiome regulator (i.e. coral species that usually maintain a constant microbiome) *P. verrucosa* (Adonis (treatment), df = 1, F = 2.3498, Pr (> F) 0.021). Specifically, the bacterial community of probiotic-treated corals only differed from placebo-treated corals after the last inoculation period (T3) (Adonis, df = 1, F = 4.2909, Pr (> F) 0.002) and not at the other sampling times (Fig. 2A-D). Similarly, in T3, probiotic-treated corals showed a significant increase in alpha-diversity metrics compared to placebo-treated corals: (Richness (S), Shannon (H'), Simpson, and Chao1 (Wilcox, p-values = < 0.0001; 0.0028; 0.0079; < 0.0001, respectively) (Fig. 3A) and enrichment of three inoculated genera (Fig. 3B). Pairwise comparisons between BMC-phylotypes in probiotic-treated and control samples revealed significant increases in the relative abundance of *Halomonas* and *Pseudoalteromonas* in probiotic-treated corals compared to placebo-treated corals (Wilcox, p-values 0.04 and 0.001, respectively). However, *Cobetia* was not detected in T3 in either treatment, while the genus *Sutcliffiella* was not detected at any sampling time. Nonetheless, *Bacillus*, the former taxonomic classification of *Sutcliffiella*, was also used as a proxy for this genus (see methods), and was significantly enriched in probiotic-treated corals (Wilcox, p-value 0.03) (Fig. 3B). In addition, the coral microbiome changed over time (Adonis (sampling time), df = 3, F = 4.5125, Pr (> F) 0.001) (Fig. S1), and the interaction between treatment and sampling time was not statistically significant (Adonis (treatment*sampling time), df = 3, F = 1.6347, Pr(> F) 0.055).

To further explore the compositional differences, the main bacterial community groups at sampling time T3 were investigated. We observed differences in the relative abundance of several groups of the dominant taxa between probiotic-treated and placebo-treated corals at T3. Overall, 4 of the 10 most abundant families were significantly enriched in the probiotic-treated corals: Rikenellaceae, Prevotellaceae, Lachnospiraceae and Rhodobacteraceae (Wilcox, p-values 0.0001, < 0.0001; < 0.0001 and 0.00042, respectively). On the contrary, the relative abundance of Endozoicomonadaceae decreased in probiotic-treated corals (Wilcox, p-value 0.0068) (Fig. 4A); other families like Simkaniaceae, Burkholderiaceae, Unclassified Alphaproteobacteria, Spirochaetaceae, and Phycisphaeraceae did not significantly differ between treatments (Table S2). Results revealed a high number of differentially abundant ASVs between probiotic-treated compared to placebo-treated colonies affiliated to several taxonomic groups (ANCOM-BC2, n = 1175 ASVs, p-adj. < 0.05) that were enriched (n = 245) or decreased (n = 930) in probiotic-treated colonies (Fig. 4B). The most enriched (p-adj. values < 0.01, W statistic > 4) and decreased ASVs (p-adj. values < 0.01, W statistic < −4) mainly belonged to the phylum Proteobacteria (n = 9, respectively), and included genera such as *Endozoicomonas* (n = 4, respectively), *Salinarimonas* (n = 1, enriched), *Delftia* (n = 1, enriched), MND1 (n = 1, enriched; n = 2, decreased) and *Catenococcus* (n = 1, decreased). Other groups from the phyla Firmicutes, Nitrospira, Bacteroidota and Verrucomicrobiota also included differentially abundant ASVs (Fig. 4C). Interestingly, ASVs enriched in probiotic-treated corals showed consistent relative abundances across biological replicates (Fig. 5). Additionally, other enriched ASVs (ANCOM-BC2, p-adj. < 0.05, W statistic ranging from 2 to 4) included potentially beneficial groups
such as members of Rhodobacteraceae (i.e. *Ruegeria*), Lactobacillaceae (i.e. *Limosilactobacillus*), *Desulfovibrio* (Desulfovibrionaceae), *Butyribrio* (Lachnospiraceae), *Ruminococcus* (Oscillospiraceae) and *Rhodococcus* (Nocardiaceae). Unclassified ASVs are detailed in Table S3. Additionally, we evaluated changes in significant ASVs (ANCOM-BC2, *p.adj.* < 0.01) affiliated with the family Vibrionaceae, as it includes opportunistic coral pathogenic bacteria. The results revealed a significant decrease in ASVs among probiotic-treated colonies, including *Photobacterium*, *Vibrio*, Unclassified Vibrionaceae, and *Catenococcus* (Fig. 4D).

In contrast to the changes observed in corals, the microbiomes associated with the surrounding seawater and sediment were not affected by the probiotic inoculation when comparing samples collected near coral colonies before (T1) and after (T3) the probiotic inoculation (see details in methods). More specifically, the seawater bacterial community did not differ between seawater surrounding probiotic-treated and placebo-treated coral colonies but changed over time (*Adonis* (treatment) *df* = 1, *F* = 2.1104, *Pr* (> *F*) 0.105; *Adonis* (sampling time) *df* = 1, *F* = 31.444, *Pr* (> *F*) 0.001) (Fig. 6A). Similarly, the bacterial community in the sediments was not significantly changed by treatment and remained stable over time (*Adonis* (treatment) *df* = 1, *F* = 1.4124, *Pr* (> *F*) 0.055; *Adonis* (sampling time) *df* = 1, *F* = 1.1946, *Pr* (> *F*) 0.181) (Fig. 6B). According to nMDS ordination, bacterial communities of coral, seawater, and sediment were distinct, forming three different groups (Fig. S2).

**Coral physiology**

The photosynthetic efficiency of Symbiodiniaceae and the thermal tolerance were also monitored, although no bleaching event or disease affected the studied corals during the experiment. Thus, putative benefits of a probiotic treatment could not fully be exerted. Photosynthetic efficiency was measured *in situ* using a diving PAM (*F*_v/_F*_m). We did not observe any treatment effect on the coral performance (Fig. S3A, Table S4). Although all corals were healthy at all sampling points, significant changes in the *F*_v/_F*_m* rates were observed over time (*p*-value < 0.001) (Table S5). The only case where we did not detect differences was between T1 and T4 (*p*-value = 0.38).

Standardized thermal tolerance thresholds (ED50s) of *P. verrucosa* were evaluated experimentally at each sampling time using the Coral Bleaching Automated Stress System (CBASS) (see details in method section). Similar to the coral fitness results, the *P. verrucosa* thermal threshold changed over time points with maximum ED50 values measured in T1 (38.06 ± 0.65) and minimum values in T4 (35.48 ± 0.7) (*p*-value < 0.001), with significant changes between time points observed (except between sampling times T2 and T3 (*p*-value = 0.71, Table S6), but not between treatments at any given time point (Fig. S3B, Table S7).

**Discussion**

Here we demonstrate that probiotics can be incorporated by corals *in situ* without causing changes in the microbial communities of the surrounding environment. Continuous probiotic application led to shifts in
the taxonomic composition and diversity of the microbiome of *P. verrucosa*, which has been previously shown to be very resistant to changes in their microbiome.

The bacterial community of *P. verrucosa* was dominated by the family Endozoicomonadaceae across seasons and treatments. However, the probiotic treatment led to a decrease in the relative abundance of Endozoicomonadaceae, possibly due to the increase of other bacterial groups. Endozoicomonadaceae are typically associated with healthy corals and often dominant in the *P. verrucosa* microbiome. Their abundance tends to decrease during thermal stress and bleaching. Nonetheless, recent studies suggest that different dominant species of Endozoicomonadaceae may have distinct roles and respond differently to local environmental fluctuations, and can also be associated with bleached corals. As we did not observe any negative effect on the coral health upon the probiotic inoculations (discussed later), we argue that the decrease in Endozoicomonadaceae was not detrimental for the coral holobiont, and may have led to an enrichment of other key bacterial groups (detailed hereafter) that could increase the holobiont resilience in the event of environmental impacts. For instance, in addition to the enrichment of inoculated genera that were previously validated as beneficial for corals (i.e. *Halomonas*, *Bacillus*, and *Pseudoalteromonas*) families including Rikenellaceae, Prevotellaceae, Lachnospiraceae, and Rhodobacteraceae were also enriched in probiotic-treated corals. Some of these families have been consistently associated with healthy hard corals, such as Rikenellaceae and Prevotellaceae, and even found in healthy soft corals (order Alcyonaceae) (e.g. Lachnospiraceae). Rhodobacteraceae is a common member of coral microbiomes and plays roles in nitrogen cycling, toxic compound degradation, and antimicrobial activity; they are early colonizers of coral larvae mucus, suggesting their role in symbiotic relationships during the early stages of coral development.

Furthermore, ASVs significantly enriched in probiotic-treated corals are potentially beneficial for the coral holobiont. Some examples include *Simkania* (Simkaniaceae), a coral endosymbiont occurring in close association with *Endozoicomonas* bacteria; *Delftia* (Commamonadaceae), a key member of coral microbiomes that plays roles in anti-quorum sensing and antibiofilm activity and may help to control pathogenic microbes associated with bleaching; and *Ruegeria* (Rhodobacteraceae), a known beneficial bacteria for multiple roles including antimicrobial effects against coral pathogens, colonization of early life stages of coral, and degradation of toxic compounds. We also observed the enrichment of fermentative bacteria such as *Limosilactobacillus* (Lactobacillaceae), formerly classified as *Bacillus*, which has been isolated from healthy coral mucus and can form stable associations with *Lactobacillus* bacteria, which are known for their probiotic activity. Other enriched fermentative bacteria included Rikenellaceae RC9 gut group, *Saccharofermentas*, *Ruminococcus*, *Pseudobutyrivibrio*, *Butyrivibrio*, and Christensenellaceae R-7 group, which may play roles in carbon metabolism and nitrogen cycling within the coral holobiont. They potentially contribute to the interplay of bacteria and Symbiodiniaceae metabolic interactions by degrading complex carbohydrates (i.e. starch) produced by Symbiodiniaceae. Enriched groups also included bacteria involved in nitrogen cycling, such as *Rhodococcus* (Nocardiaceae) which are known for their role as nitrogen-fixing symbionts and
antimicrobial activity \textsuperscript{71,72} and degradation of emergent contaminants \textsuperscript{73} such as endocrine-disrupting compounds prevalent in the marine environment \textsuperscript{74}. Other nitrifiers include Mle-1-7 group (Nitrosomonadaceae) and \textit{Nitrospira} (Nitrospiraceae), which may be important for the primary productivity of coral photosynthetic symbionts by making nitrogen compounds available \textsuperscript{75}. We also observed enrichment of protozoan coral intracellular endosymbionts such as \textit{Candidatus} Amoebophilus \textsuperscript{76–78}, which interact with eukaryotic hosts such as \textit{Symbiodinium} spp. and apicomplexans \textsuperscript{79,80}.

Moreover, we observed a decrease in potential pathogenic bacteria from the family Vibrionaceae, from which some members constitute opportunistic coral pathogens contributing to coral diseases and bleaching \textsuperscript{81–90}. We observed that the probiotic treatment led to a decrease in the abundance of \textit{Vibrio}, Unclassified Vibrionaceae, \textit{Photobacterium}, and \textit{Catenococcus}. Previous studies have also shown that probiotics can reduce \textit{Vibrio} abundance and mitigate coral bleaching \textsuperscript{13,14}. These findings suggest that cumulative \textit{in situ} probiotic inoculations may contribute to a beneficial restructuring of the coral-associated bacterial community, signified by the decrease in specific coral pathogens, which may be advantageous during thermal stress, potentially preventing or minimizing opportunistic pathogenic infections in the coral holobiont \textsuperscript{11}.

We also explored the aforementioned presence and relative abundance of the BMC genera in the coral microbiome. We observed an overall enrichment of \textit{Halomonas} and \textit{Pseudoalteromonas} in probiotic-treated corals in T3, which may lead to an increase in beneficial microbial functions in the coral holobiont \textsuperscript{13,14}. Although we did not detect the presence of the BMC \textit{Sutclifiella}, the current 16S amplicon data pose difficulties in the taxonomic resolution of recently re-classified groups, such as BMC \textit{Sutclifiella}, a novel genus previously classified as \textit{Bacillus} and recently described \textsuperscript{91}. Nonetheless, we observed enrichment of \textit{Bacillus} in probiotic-treated corals in T3, although there is no certainty of which specific \textit{Bacillus} ASVs correspond to \textit{Sutclifiella} in the coral microbiome. Previous studies found evidence of successful BMCs incorporation and further enrichment in the coral microbiome upon microbiome manipulation in controlled experiments \textsuperscript{13,14,34,37}. In this context, despite the low microbiome flexibility described for the \textit{Pocillopora} genus \textsuperscript{42}, prokaryotes can also be acquired from the environment \textsuperscript{49,65,92}, which may have allowed the BMCs incorporation and enrichment. BMCs genera were found in very low abundance in the native microbiome of the studied \textit{P. verrucosa}, but their relative abundance was significantly increased in T3, suggesting their incorporation and/or enrichment upon frequent probiotic inoculations, even in corals that were not under stress. On the contrary, the enrichment of probiotics seems to be facilitated when corals are under stress \textsuperscript{14}, which may indicate that, if the goal is the rehabilitation and retention of threatened corals, the use of probiotics as medicine applied in times of stress (for example, following the threatened areas indicated by the National Oceanic and Atmospheric Administration, NOAA bleaching alert system) may be a good strategy to be tested \textsuperscript{10,93}. Furthermore, the dominance of certain bacteria, (i.e. Endozoicomonadaceae) and the low flexibility of the host microbiome \textsuperscript{47} may also influence this process. The use of dominant symbiotic bacteria (i.e. \textit{Endozoicomonas} sp.) may represent a promising strategy for a faster and perhaps more stable enrichment \textsuperscript{94}; however, without additional inoculations.
their retention may be ultimately subject to environmental changes and other variables that can influence the coral-associated microbiome\textsuperscript{95–98}.

At T4, five months after the last inoculation, the bacterial community shifted back towards pre-inoculation bacterial profiles, which aligns with previous findings in corals\textsuperscript{14} and other organisms where the effect of probiotics ceases once the probiotic administration is suspended\textsuperscript{99}. Hence, this data provides evidence of such a probiotic effect \textit{in situ} in coral-associated bacteria, evidencing that frequent inoculations can temporarily trigger microbiome restructuring. The necessary frequency of inoculations (weeks, months, years) and probiotic cell concentration may depend on different variables, such as the goals and environmental conditions, and need further investigation. Other factors such as BMCs consortium composition, coral species, multi-host vs. single-host bacterial donors, host-microbiome flexibility, and host health (i.e. bleaching state, diseases) likely influence host microbiome-probiotic interactions\textsuperscript{10,11}. The potential mechanisms underlying probiotic action in the coral microbiome are likely multi-factorial\textsuperscript{100}, and may include the enrichment of beneficial bacteria, indirect niche colonization by inoculated BMCs, antagonistic effects against pathogens\textsuperscript{13}, predatory bacteria\textsuperscript{101}, immune stimulation by allochthone strains triggered by the probiotic\textsuperscript{14,102}, and support for coral heterotrophic feeding\textsuperscript{35}. Further studies are required to better understand these mechanisms and improve microbiome rehabilitation. Unraveling genomic-level mechanisms associated with BMCs and using omics to track the actual changes promoted by probiotics are crucial steps for addressing knowledge gaps on the functioning of coral probiotics\textsuperscript{100}, as recently investigated\textsuperscript{14,19,39}, and would improve the selection of BMCs based on targeted microbial traits.

We assessed the \textit{in situ} photosynthetic efficiency of Symbiodiniaceae and thermo-tolerance response in CBASS experiments as indicators of coral holobiont health. We did not observe any significant changes between treatments in these health indicators at any of the sampling times, perhaps due to the overall healthy profile of the colonies throughout the experiment. These results may suggest that probiotics do not seem to cause harm if there is nothing to be fixed, although they still provide a microbiome (and, potentially, epigenomic)\textsuperscript{36} restructuring that could be beneficial in times of stress. Further \textit{in situ} experiments should investigate the effects of these inoculations in the event of a bleaching event or disease outbreak, and/or the effects of probiotics on diseased or thermally stressed corals displaying signs of bleaching, which would provide insights into the effects of coral probiotics in coral hosts with various health statuses.

The significant changes observed in the coral microbiome and lack of significant effects on the bacterial communities associated with sediments and seawater suggest both the prominent targeted effect of coral probiotics and the absence of potential impacts on the environment. The seawater bacterial community displayed more variability over time, while the sediment bacterial community remained stable across sampling times. Coral reefs harbor heterogeneous microbial communities in different niches within the ecosystem\textsuperscript{103–108}. It is widely known that the surrounding microbiomes are distinct from the coral microbiome and exhibit different bacterial community profiles with differential functionality\textsuperscript{42,109–}
Our findings provide the first evidence that coral probiotic bacteria can be incorporated in situ and do not induce changes in the surrounding coral microbiomes, supporting the potentially safe and feasible application of probiotics in reef ecosystems.

**Materials and methods**

**BMC isolation from healthy corals**

Coral fragments from *Stylophora pistillata* Clade IV, *Galaxea fascicularis* and *Pocillopora verrucosa*, were collected by snorkeling and Scuba diving at Thala reef (22°15′46.9″N 39°03′05.9″E), Aquarium (22°23′15.6″N 38°55′07.2″E) and Al Fahal reef (22°18′18.4″N 38°57′52.5″E) respectively, in the central Red Sea, at depths of 1–10 m, between February and May 2021. Coral fragments were collected by Scuba diving using gloves and pliers, and transported in 50-ml conical tubes on ice for approximately 1 hour to the laboratory. Immediately on arrival, the fragments were macerated using 1–2 mL of 3.5% sterile saline solution with a sterile mortar and pestle. Serial dilutions up to $10^{-6}$ were performed using the macerated paste with 3.5% saline solution, and 100 µL of each dilution was plated in Marine Agar (MA) (Zobell 2216, HiMedia Laboratories, Mumbai, India), adjusted to 3.5% salinity, diluted Marine Agar (Marine Agar Medium 2× diluted to 3.5% NaCl) (DMA), and Luria Bertani agar (LB) (Sigma-Aldrich®), adjusted at 3.5% salinity. Plates were incubated at 25°C (corresponding to the in-situ water temperature registered at the sampling sites at the moment of fragments collection) overnight. In parallel, the coral macerate was incubated in 50 ml of 3.5% saline solution in a 250 mL sterile Erlenmeyer at 27°C overnight with glass beads at 130 rpm. After this first incubation, triplicate subsamples (100 µL) of $10^{-4}$, $10^{-5}$, $10^{-6}$ dilutions were plated into MA and DMA culture media and incubated under the same conditions described above. Additionally, 0.5 cm coral fragments were placed on the Petri dishes containing MA and DMA. All the plates were incubated at 25°C for at least 48 h or until visible bacterial colonies were observed. Approximately 350 bacterial isolates were obtained, based on colony morphology, and were preserved at -80°C using sterilized glycerol with a final concentration of 20%, for further analysis.

**Bacterial genomic DNA extraction and 16S rRNA gene sequencing of bacterial isolates**

Each bacterial isolate from the glycerol stocks was re-grown using 200 µL of the stock and inoculated into 6 mL of Marine broth (HiMedia Laboratories, Mumbai, India), and incubated overnight at 26°C with 140 rpm agitation. For bacterial DNA extraction, 2 ml of bacterial liquid culture were centrifuged for 5 min at 10.000 rpm to obtain a pellet and washed twice with 3.5% saline solution to wash the cells from the culture media. The DNA extraction was performed using the Wizard® Genomic DNA purification kit (Promega Corporation, USA), following the protocol for Gram-positive and Gram-negative bacteria. Genomic DNA was purified with the GFX™ PCR DNA and Gel band purification Kit (Cytiva Company, USA) and then quantified using Nanodrop™ 8000 Spectrophotometer (Thermo Scientific™) and Qubit™ dsDNA broad range assay kit (Invitrogen™). To target the full 16S rRNA gene, universal primers 27F
5’AGAGTTTGATCMTGGCTCAG 3’ and 1492R 5’ GGTTACCTTGTTACGACTT 3’ were used, using the AmpliTaq Gold® 360 Master Mix (applied Biosystems®, by Life Technologies™) under the following PCR conditions: one cycle of initial denaturation at 95°C for 5 min, 30 cycles of denaturation at 95°C for 1 min, annealing at 50°C for 1 min, extension at 72°C for 1 min, and one cycle of final extension at 72°C for 7 min. The amplification was verified using 1% agarose gels (100 V, 40 min) and visualized in a Bio-Rad® transilluminator. PCR products were sent to Macrogen (Korea) for taxonomic identification by Sanger sequencing. The forward and reverse sequences (1000–1500 bp) were processed to remove low-quality bases and generate contigs using the ChromasPro software. Ambiguities in the assembled sequences were resolved visually (either by choosing the base from the read with the cleaner signal or changing the consensus base to “N”). Cleaned assembled DNA sequences from each of the BMC isolates were then identified using the EzBioCloud server. The top-hit taxon, obtained from average nucleotide identity (ANI), was used to estimate the taxonomy of the BMC isolates.

**Functional screening of bacterial isolates**

Bacterial strains identified as potential human or coral pathogens (e.g. *Vibrio* spp.) were excluded (n = 305). The remaining bacterial isolates were tested for beneficial functions, following previous studies. For reactive oxygen species (ROS) scavengers, 20 µL of pure culture of each bacterial strain was placed on a portable microscopy slide, and a drop of 3% (v/v) of hydrogen peroxide was immediately added in the center. The criterion of a positive result was evaluated qualitatively, based on the production of bubbles, as a proxy of catalase reaction. Phosphate assimilation was tested according to Nautiyal, 1999 using Pikovskaya’s agar culture media (HIMEDIA®): 20 µL of pure culture of each strain was dispensed onto the media plate. As bacterial growth occurred overnight, strains that were positive for phosphate assimilation produced a transparent halo around the cultures. The antagonistic effect against *Vibrio corallilyticus* (a well-known coral pathogen) was assessed through the diffusion agar method: first, 20 µl of each BMC bacterial strain was spot-inoculated onto 2.5% NaCl LB agar, placing three spots for each strain (representing replicates). The plates were incubated at 26°C for as long as necessary to allow the strain to grow. The strains were inactivated by chloroform volatilization, followed by pouring 3 ml of semisolid 2.5% NaCl LB medium (0.7% agar) containing the strain *Vibrio corallilyticus* BAA-450 indicators over the inactivated spots. These plates were incubated at 28°C for 16 h, and the antagonistic activity was indicated by inhibition halos around, or no detection of *Vibrio* growth over, the colony spot.

**Selection of BMCs and probiotic preparation**

Six bacterial strains (two *Pseudoalteromonas galatheae* and two *Cobetia amphilecti* isolated from *P. verrucosa*; one *Halomonas* sp. isolated from *Stylophora pistillata* and one *Sutcliffiella* sp. isolated from *Galaxea fascicularis*) were chosen for the probiotic consortium, based on their beneficial traits (Table S1). As the probiotic consortium is composed of a diverse combination of bacteria, each strain was collected proportionally at the peak of the exponential growth phase, to achieve a concentration of $1 \times 10^9$ cells/ml.
per strain. Fresh bacterial cultures were collected and washed three times using saline solution (3.5% NaCl) by centrifuging at 6000 g for 5 min each time, followed by resuspension in 1 L of saline solution (3.5% NaCl) for a final concentration of approximately $10^9$ cells/ml. The probiotic was stored at 4°C for a maximum of one week until in-situ inoculation in the corals.

**Experimental design**

The study site was located in a shallow sheltered area in “Al Fahal Reef” (22°18’18.4”N; 38°57’52.5”E), a mid-shore reef in the central Red Sea, 15 km off-shore from King Abdullah University of Science and Technology (KAUST), Saudi Arabia (Fig. 1B). The experiment was performed at “The Red Sea Research Center Coral Probiotic Village”, a multidisciplinary research initiative established to test the use of coral probiotics and other pioneering strategies in situ in real coral reef setups. It covers an area of about 500 m$^2$, with a maximum depth ranging from 8 to 10 m. In the study area, 30 visually healthy colonies (no visual signs of bleaching or disease) of the brown morphotype of *P. verrucosa* were selected for the experiment (with a minimum distance of 3 m between colonies to minimize sampling of clonal genotypes), and were randomly assigned to the probiotic and control (referred to here as placebo) treatments ($n = 15$ colonies per treatment). The health status of colonies was qualitatively evaluated throughout the experiment, using a coral health chart to assess signs of bleaching. The study was performed from summer to winter 2021 and late spring 2022 to encompass seasonal variations. Four sampling points were considered for analysis: T1, before the treatment inoculations (late August 2021); T2, after one and a half months of inoculations (mid-October 2021); T3, at the end of the inoculations (late November 2021); and T4, five months after the last inoculations (April 2022). Inoculations were performed repeatedly with a frequency of three times per week during a three-month period (T1-T3), using 50 ml plastic syringes containing 30 mL of the probiotic consortium (with a final concentration of $1 \times 10^9$ cells/mL), released slowly over the coral colony (Fig. S4). The placebo treatment consisted of an autoclaved 3.5% NaCl solution (the same used to resuspend BMC-cells) applied in the same way. The use of inert negative controls (i.e., without the addition of any confounding factors), is the gold standard procedure for testing probiotics, as described in Garcia-Bonet et al (2023) $^{100}$. Dead cells should not be used as a negative control as they can also trigger specific responses in the inoculated hosts and are, therefore, not inert $^{100}$. Fragments from each colony were collected for coral-associated bacterial community analysis by Scuba diving at all sampling times (T1-T4) using sterile gloves and pliers (one for each treatment) and individual sterile collection bags (Whirl-Pak ®). On the boat, immediately after collection, coral fragments were placed in sterile 5 mL cryovials and covered with DESS buffer (20% dimethyl sulfoxide (DMSO), 0.25 M ethylenediaminetetraacetic acid (EDTA) and saturated sodium chloride (NaCl), with adjusted pH 8.0), and immediately snap-frozen in liquid nitrogen. Samples were transported to the laboratory (less than 3 h after collection) and stored at -80°C until further processing. In parallel, sediments and water surrounding the coral colonies were collected at T1 and T3 to monitor their bacterial communities and assess water nutrients and dissolved organic carbon (DOC). The surrounding water and sediments from 10 of the studied *P. verrucosa* colonies distributed in different areas of the experimental study site ($n = 5$ per treatment) were sampled (using a random number generator): sediment
samples were collected at the bottom of each colony between 1–5 cm depth approximately, using sterile 50 mL falcon tubes. Water samples were collected approximately 30 cm distant from the same colonies, using 2 L dark bottles that were acid-washed in HCl 4% for 10 min prior to the sample collection. Samples were stored on ice on the boat and filtered the same day upon arrival in the laboratory, using a filtration rack with 0.22µm Millipore Sigma membranes attached to a vacuum pump. Filter membranes were individually stored at -80°C, for less than two weeks, until DNA extraction. All equipment and materials used were thoroughly sterilized to avoid contamination. All sampling procedures were carried out within a one week interval for each sampling time (T1-T4). During T1-T3, samples of coral, seawater and sediment were collected for microbial community analysis on the same day, immediately before starting the placebo and probiotic inoculations.

**Monitoring of in-situ physicochemical parameters and inorganic nutrients**

Seawater temperature and salinity were monitored throughout the duration of the experiment using multiparameter CTDs (Ocean Seven 310 Multiparameter CTD, Idronaut). The daily minimum, maximum and mean seawater temperature and mean salinity values are summarized in Table S8. Seawater collected during T1 and T3 was used for inorganic nutrient analysis of the surrounding water of each of the randomly selected coral colonies (n = 5 per treatment). Briefly, water was filtered on the boat, with 0.22µM Millex®-GV filters (PVDF Membrane, Merck Millipore Ltd., Ireland), into 15mL falcon tubes. Subsequently, samples were placed on ice on the boat and then frozen at -20°C until analysis. The inorganic nutrients analyzed were: Silica (Si(OH)₄), Nitrite (NO₂⁻), Nitrate (NO₃⁻), and Phosphate (PO₄³⁻). All measurements were performed using a segmented flow analyzer (Model AA3 HR, SEAL Analytical Inc.) with the following detection limits: Silicates 0.08322 µmol.L⁻¹; Nitrate 0.0217 µmol.L⁻¹; Nitrate 0.0322 µmol.L⁻¹; and Phosphate 0.01052 µmol.L⁻¹ (Table S8).

In-situ photosynthetic efficiency for coral health monitoring

The photosynthetic efficiency of the algae symbionts (Symbiodiniaceae) was assessed through the maximum quantum yield of PSII photochemistry $F_{v}/F_{m}$. A pulse-amplitude modulation (PAM) diving-PAM system (Diving PAM II, Walz) with a red-emitting diode was used (LED; peak at 655 nm). PAM data was collected after sunset, at least 30 min after complete darkness, to ensure there was full photochemical dissipation of the reaction centers. The diving PAM was configured as follows: measuring light intensity = 6; gain = 2; and damping = 4.

The changes in photosynthetic efficiency ($F_{v}/F_{m}$) over time in different treatments were analyzed using a linear mixed effect model using the function “lmer” from R package lme4 in R studio (R Core Team). Colony (biological replicates, n = 15) nested to treatment was treated as a random effect on the intercept to account for the non-independence of replicates with time. $F_{v}/F_{m}$ was included in the model as a response variable, sampling time as a predictor variable, and treatment as a factor with two levels: probiotic and placebo. We performed model selection using likelihood-ratio tests starting with the most
Changes in the $F_v/F_m$ over time were tested using the “emmeans” R function in all pairwise combinations.

Coral Bleaching Automated Stress System (CBASS) experiments

To evaluate coral heat response behavior during the experiment, short-term acute heat stress assays were performed to determine the coral thermal threshold at the genotype (per-colony) level. Here, CBASS was used as a proxy to assess coral health and determine if the long-term inoculation of coral probiotics had an effect on coral thermal tolerance threshold, as well as their natural thermotolerance variation during a seasonal time frame. In sampling times T1, T2, T3, and T4, four fragments of each colony were collected. The fragments were transported in seawater to the wet lab facility of the Coastal and Marine Resources Core Lab (CMR, KAUST), where the set-up was ready to receive the corals. Briefly, the system consists of four 10L flow-through supplied with raw seawater collected from the site a day before the runs. Each tank runs different temperature regimes independently and the light setting was adjusted to correspond to $in-situ$ irradiance (600 µmol photons m$^{-2}$ s$^{-1}$), which was adjusted using an LI-193 Spherical Underwater Quantum Sensor (LI-COR) and manual adjustment of dimmable 165W full spectrum LED aquarium lights (Galaxyhydro). The lights followed a 12:12 h day/night cycle. The temperature of each tank was controlled using the ITC-310T-B (Inkbird) thermostat connected to an IceProbe Thermoelectric chiller (Nova Tec) and 200W titanium aquarium heaters (Schego). HOBO Pendant® Temperature Data Loggers (Model UA-001-64) recorded the temperature of each tank every 10 min during the experiment. One fragment corresponding to each colony was exposed to a different temperature condition. The temperature regime of each tank was 1 control/baseline − 30°C, 1 medium − 33°C, 1 high − 36°C, and 1 extreme − 39°C. The CBASS assays ran for 18 h, where the temperature of the 30°C tank was maintained at 30°C for the entire experiment; in the other tanks, the temperature was increased to 33°C, 36°C, and 39°C, respectively, and then returned to 30°C overnight until the end of the experiment. The detailed temperature profiles are provided in the supplementary material (Table S9). After 7 h from the start of the experiment (and one hour in darkness), we measured the endosymbiotic algae photosynthetic efficiency ($F_v/F_m$) for all fragments using a pulse-amplitude modulation (PAM) fluorometry (Diving PAM II, Walz). The measurement also matched the temperature ramping down to 30°C.

The data were analyzed according to Voolstra and collaborators (2020)\textsuperscript{43}, where $F_v/F_m$ values were used to evaluate the treatment’s ED50, corresponding to its thermal threshold. ED50 corresponds to the effective doses that cause a 50% decrease in the $F_v/F_m$. The dose-response curves were fitted using the R package “drc”\textsuperscript{121}. The changes in ED50 with sampling time at different treatments were analyzed using a linear mixed effect model using the function lmer from R package “lme4”\textsuperscript{120} in R studio (R Core Team). Colony (biological replicates, n = 15) nested to treatment was treated as a random effect on the intercept to account for the non-independence of replicates with time. $F_v/F_m$ was included in the model as a response variable, sampling time as a predictor variable, and treatment as a factor with two levels: probiotic and placebo. Model selection was performed using likelihood-ratio tests starting with the most
complex model and sequentially removing terms until all parameters were significant at $p < 0.05$. Changes in the ED50 over time were tested using the “emmeans” R function in all pairwise combinations.

**DNA extraction, library preparation, and sequencing of bacterial communities**

The DNA from the coral fragments was extracted using a DNeasy® Blood & Tissue kit (Qiagen) according to manufacturer instructions, with the Gram-positive bacteria pre-treatment and the following modification: coral fragments of approximately 0.5 g were used directly for the extraction. The lysis incubation step after adding proteinase K was carried out overnight for approximately 16 h at 56°C, with a constant agitation at 650 rpm in a Thermomixer (ThermoFisher®).

DNA was extracted from water samples using a DNeasy® Blood & Tissue kit (Qiagen) by cutting the filter into small pieces with a sterile cutter and tweezers. The protocol was performed following manufacturer instructions, with the following modifications: at the sample pre-extraction preparation stage, half of the membrane filter was cut into smaller pieces and then placed in 1.5mL microcentrifuge tubes. The volume of all the following solutions used in the kit was adjusted to similar proportional volumes (thus not changing any concentration of compounds) to fully immerse all the membrane filter pieces into the solution: 540µL ATL buffer and 60µL Proteinase K were added. After adding these solutions, the incubation step at 56°C was conducted for three hours. Then, the volumes of buffer AL and ethanol were 400µL, buffer AW1 and AW2 were 500µL, and the final elution buffer AE was 50µL. DNA samples were stored at -20°C until downstream analyses.

DNA was extracted from sediment samples using a DNeasy® PowerSoil Kit (Qiagen), with the following modification: 12.5 µL of Proteinase K was added to approximately 0.5 g of sediments for incubation overnight at 56°C, with constant agitation at 650 rpm in a Thermomixer. The downstream steps were performed according to the kit's protocol. DNA concentration and purity for all samples (coral, water, and sediment) were quantified using a Qubit™ dsDNA assay kit (Invitrogen™) and Nanodrop™ 8000 Spectrophotometer (Thermo Scientific™). Sequencing of the V3-V4 regions of the 16S rRNA gene was performed using the universal primers 341F 5’ CCTACGGGNGGC WGCAG 3’and 785R 5’ GAC TAC HVG GGT ATC TAA TCC 3’ for the coral, sediment, and water samples, at Novogene Corporation-Inc in China. In brief, PCR mixtures contained 15 µL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 0.2 µM of each of the forward and reverse primers, and 10 ng of the samples’ genomic DNA. The thermal cycling conditions were as follows: a first denaturation step at 98°C for 1 min, followed by 30 cycles at 98°C for 10 s, 50°C for 30 s, and 72°C for 30 se, and a final extension of 5 min at 72°C. PCR products were verified and quantified by mixing their equal volume with 1X loading buffer (contained SYB green) and performing electrophoresis on 2% agarose gels. For the library preparation, PCR products were purified using a Qiagen Gel Extraction Kit (Qiagen, Germany). Sequencing libraries were generated with a NEBNext® Ultra™ II DNA Library Prep Kit (Cat No. E7645). The library quality was evaluated on a Qubit® 2.0 Fluorometer (Thermo Scientific™) and Agilent Bioanalyzer 2100 system. Libraries were sequenced on a NovaSeq platform (Illumina) and 250 bp paired-end reads were generated.
All sequence reads were deposited in the European Nucleotide Archive (ENA) under the study accession number PRJEB65896.

**Bacterial community analyses.**

The DADA2 pipeline was used to infer Amplicon Sequence variants (ASVs) using the 16S rRNA gene-based amplicon libraries of coral, sediment, and water. Briefly, the raw reads were decontaminated of phiX, and adapter-trimmed using the “BBDuk” tool from the BBMap suite (Bushnell B, http://sourceforge.net/projects/bbmap/). PCR primers were then removed from the reads using the “cutadapt” tool. After performing concatenation of the forward and reverse reads via “justConcatenate” option in the “mergePairs” function of DADA2, the sequences were analyzed under the pseudo-pooling mode by following the standard DADA2 (version 1.22) workflow and using the SILVA database, version 138.1. The potential contaminant ASVs that were identified in the negative controls and the study samples were removed from the analysis by the “decontam” tool using the prevalence-based method (on the default threshold setting). ASV raw counts are available in the Zenodo repository.

In brief, reads corresponding to mitochondria, chloroplast, archaea, eukaryotes, and singletons were removed, resulting in 46803 ASVs for the coral dataset, 34099 for the water dataset, and 68354 for the sediment dataset. Alpha and beta diversity, plots, ordinations, and statistical comparisons were carried out in R version 4.2.2 (R Core Team, 2018) using the functions in Phyloseq version 1.42.0 and Vegan version 2.6-4. All plots were generated using ggplot2 version 3.4.0. Additional figures to represent the experimental design were created in a licensed version of BioRender. Alpha diversity of the coral bacterial community was calculated using the rarefied ASV counts to the minimum sample depth (18364 reads) with the function “estimate.diversity” from Phyloseq, with the default diversity indices (Observed S, Shannon H’, Simpson, and Chao1). Statistical comparisons between treatments for alpha diversity metrics were calculated by implementing the Wilcoxon test, and previous testing of the null hypothesis for normal distribution of the data (Shapiro-Wilks, p-value < 0.05). Non-metric Multidimensional Scaling (nMDS) analyses were generated from Bray-Curtis distances of Wisconsin-square root transformed ASV total counts using the “vegdist” and “metaMDS” functions in Vegan for the coral dataset. Principal Component Analysis (PCoA) was implemented for the water and sediment datasets from Bray-Curtis distances. Statistical differences in the microbial communities of coral, seawater, and sediment components were assessed using the transformed ASV counts to relative abundance. The Permutational Multivariate Anova test (PERMANOVA) was implemented to test for significance, using “sampling time” and “treatment” as factors, implementing the “adonis2” function in Vegan, from generated Bray-Curtis distances and 999 permutations. The homogeneity of variances was calculated between treatments (placebo and probiotic) using the “betadisper” and “permutest” functions in Vegan using Bray-Curtis distances and 999 permutations for the coral, water, and sediment datasets. For the coral dataset, this was calculated for each sampling time. In T3, the variances between treatments in the coral dataset were not homogeneous (Betadisperse, df = 1, F = 7.2107, Pr (> F) 0.012). Nonetheless, as PERMANOVA is largely unaffected by heterogeneity in balanced designs, we proceeded to calculate the statistical
significance of the treatment using the *Adonis* function for T3 samples (biological replicates, placebo: n = 14; probiotic: n = 15).

Pairwise comparisons to evaluate changes in the relative abundance of the dominant bacterial families in the coral microbiome between treatments (placebo and probiotic) in T3 were carried out using the two-sided Wilcoxon test, after testing for normal distribution of the data (*Shapiro-Wilk, p-values* < 0.05). The enrichment of the BMC genera in the coral microbiome in T3 was assessed by comparing their relative abundance between treatments, using the Wilcoxon test, and previous testing for normal distribution of the data (*Shapiro-Wilk, p-values* < 0.05). The genus *Sutculifella* was not detected in the coral dataset; nonetheless, this genus was previously part of the *Bacillus* genus and was recently re-classified. The coral 16S rRNA gene amplicon data might not reflect its most recent taxonomy; therefore, we included the genus *Bacillus* as a proxy of *Sutculifella* in these comparisons.

To identify differentially abundant ASVs between placebo and probiotic treatments in the coral microbiome in T3, the Analysis of Composition of Microbiomes with Bias Correction “ANCOM-BC2” was used. This method estimates unknown sampling fractions, corrects bias from sample differences, models absolute abundance with linear regression, and provides a statistically valid test with appropriate *p*-values, false discovery rate (FDR) control, and sustained power. We performed this analysis on total ASV counts (after removing singletons), using the Benjamini-Hochberg (BH) method to correct for false positives and an alpha of 0.05 for significance. An ASV was considered significant when it was enriched or decreased significantly (*p*-adj. < 0.05) in the probiotic samples in comparison to the placebo (reference group) under the aforementioned parameters. We focused on the top 20 most enriched (*p*-adj. < 0.01, W statistic > 4) and 20 most decreased (*p*-adj. < 0.01, W statistic < 4) ASVs.

**Declarations**

**Reporting Summary**

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

**Data availability**

All sequence reads were deposited in the European Nucleotide Archive (ENA) under the study accession number PRJEB65896. Other data supporting the results of this study are provided as Supplementary data files. Large data sets will be available on Zenodo upon acceptance or request.

**Code availability**

All R code used in this study is available in Zenodo upon acceptance or request.

**Acknowledgements**
We acknowledge KAUST Core labs and CMOR staff for their technical and logistics support for laboratory processing and diving operations. This work was supported by KAUST grant number BAS/1/1095-01-01 and the KAUST Center Competitive Funding (CCF) FCC/1/1973-51-01.

**Authors contributions**


**Competing interests**

The authors declare no competing interests.

**Additional information**

All data required to perform the analyses and generate the results in this manuscript will be available from Zenodo upon acceptance or request.

**Supplementary information.** The online version contains supplementary material available at XXX.

**Peer review information**

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**References**


**Figures**
Figure 1

A) Summary of BMCs isolation and probiotic assemblage. B) Location of the study site in the Central Red Sea where in situ experimental treatments took place. C) Seawater temperature changes during the experiment sampling times. Daily mean temperatures (red dots) are indicated. The inoculation period from T1 to T3 (placebo and probiotic syringes) and all sampling times (T1-T4) are indicated. D) Summary of analyzed microbial communities (coral, seawater, and sediment near the corals) and monitoring of the coral physiology. Icons indicate the analysis conducted at each sampling time.
Figure 2

Compositional changes in the bacterial community of *Pocillopora verrucosa* associated with the *in situ* inoculation of coral probiotics. Nonmetric multidimensional scaling ordination (nMDS) of the *P. verrucosa* microbial community according to sampling time and treatment (*k* = 2) in A) T1, B) T2, C) T3, and D) T4.
Figure 3

A) Alpha diversity indices (H′ = Shannon-Weaver diversity, Simpson and Chao1) estimated by treatment (placebo and probiotic) at each sampling time are shown. The statistically significant differences are denoted with asterisks: ** = p < 0.01; *** = p < 0.001. B) Relative abundance of the BMC genera in the coral microbiome of P. verrucosa at T3 according to treatment (placebo and probiotic). Significant differences were detected between treatments in Bacillus (here as a proxy of Sutclifiella), Halomonas, and
*Pseudoalteromonas* in probiotic-treated corals. The statistically significant differences are denoted with asterisks: \(*= p < 0.05; ** = p < 0.01; *** = p < 0.001.\)

Figure 4

*Pocillopora verrucosa* microbiome restructuring after long-term probiotic inoculation (T3 sampling time).  
A) Families among the top 10 most abundant in the coral microbiome, with significant changes in relative
abundance between placebo- and probiotic-treated corals. The statistically significant differences are denoted with asterisks: \( ** = p < 0.01; *** = p < 0.001 \). B) Volcano plot depicting differentially abundant ASVs (dots) identified in the ANCOM-BC2 analysis (n = 1175 differentially abundant ASVs in probiotic- in comparison to the placebo-treated) (Data table available in Zenodo). The log fold change (X-axis) and the p-adj. (Y-axis) value for each ASV are represented. Blue dots indicate enriched ASVs and red dots indicate decreased ASVs with a q-val (adjusted p-value) < 0.05. ASVs that are not significantly different in abundance between treatments (probiotic vs. placebo) are colored in gray. C) Top differentially abundant ASVs with their associated genus taxa and color-coded by phylum. The W test statistic from the ANCOM-BC2 is shown (negative W indicates decreased taxa while positive W indicates enriched taxa in probiotic-treated corals in comparison to placebo-treated corals. “Unclassified” ASVs are available in Table S3. D) Differentially abundant ASVs affiliated with the Vibrionaceae family, colored by genera. The dot size represents the Log Fold change of each ASV.
Figure 5

Heatmap representing the relative percentage of the top 20 most enriched (top part) and 20 most decreased (bottom part) ASVs in probiotic- vs placebo-treated corals in T3. The X axis represents biological replicates (colony) for each treatment, respectively.
Figure 6

Coral surrounding bacterial communities according to treatments and time. Principal Component Analysis (PCoA) of A) seawater and B) sediment bacterial communities in the surrounding environment of placebo- (blue) and probiotic-treated (purple) corals in T1 and T3.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarytables3oct23NDFINAL.xlsx
- Supplementaryfigures.docx