Evolutionary Genomics Guides Scalable Coral Probiotics for Climate Resilience

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Abstract

Coral reefs sustain nearly 30% of marine biodiversity [1] and provide ecosystem services valued at $2.7 trillion USD annually [2], yet face catastrophic collapse under climate change [3], with 70-90% projected for extinction by 2050 even if the 1.5°C Paris target is met [4]. While microbial probiotics could enhance resilience, scalability is limited by transient colonization and uncertain long-term efficacy [5-9]. Here, we introduce an evolutionary genomics framework to select next-generation probiotics targeting bacteria undergoing irreversible transitions to host dependency. Through laboratory proof-of-principle, we show the coral-sourced *Ruegeria* MC10, identified by genomic signatures of host adaptation (insertion sequence proliferation and pseudogenization of essential metabolic genes), increases thermal tolerance thresholds by 1°C in model cnidarians. Subsequent, real-world application of one-month nursery inoculations enabled *Acropora pruinosa* corals to retain MC10 for the monitored period (eight months) post-outplanting, including persistence through a natural bleaching event, with evidence for increased resilience. This work establishes lifestyle-oriented probiotic selection as a scalable solution for microbial-driven reef restoration. Critically, this framework addresses poor probiotic colonization and limited scalability, which pose universal limitations across conservation and aquaculture that hinder microbial-based interventions [10-13]. By merging evolutionary biology with conservation science, we provide a generalizable blueprint applicable to diverse threatened wildlife.

Main Text

Coral reefs sustain nearly 30% of marine biodiversity [1] and support the livelihoods of over one billion people through ecosystem services and coastal protection [14]. Yet these vital ecosystems are collapsing under climate change: since the 1950s, half of global live coral cover has vanished, and even under the 1.5 °C Paris target, 70-90% of reefs face extinction by 2050 [4]. Central to this crisis is the breakdown of the coral-algal symbiosis during thermal stress, which triggers bleaching and starvation [15]. The accelerating frequency of mass bleaching events, including the catastrophic 2014-2017 [16] and 2023-2024 [17] global events, has left reefs with insufficient recovery intervals, necessitating urgent interventions to enhance resilience.

The targeted manipulation of microbial communities is considered a promising strategy to enhance the resilience of endangered wildlife [7], but uncertainty regarding establishment of long-term relationships remains [11]. Microbial therapies, particularly probiotics (i.e., beneficial microbes administered to improve host health), offer promise for mitigating coral bleaching by modulating holobiont health. Laboratory studies demonstrate that probiotic inoculations can enhance thermal tolerance, disease resistance, and calcification [8,18,19], while field trials confirm their safe delivery with limited evidence for off-target effects [5].

To address the issue of long-term residency of bacterial inoculants, we introduce so-called next-generation probiotics selected through evolutionary principles. By targeting beneficial bacteria undergoing irreversible transitions from free-living to host-dependent symbiosis, we identify microbes pre-adapted for stable colonization. We focus on evolutionary young endosymbionts, which retain culturability but exhibit genomic adaptations (e.g., pseudogenization of core metabolic genes) indicative of host dependency, assumed to align with more persistent association in the tissue. Through screening of 1,212 bacterial isolates from

corals, we identified a novel population taxonomically affiliated with *Ruegeria*, a genus ranked among the most prevalent coral-associated taxa [20,21] with a coral-sourced isolate recently shown to be a coral probiotic [22], that exhibits genomic hallmarks of host adaptation. Nursery inoculations enable MC10 to persist in coral tissues throughout an 8-month field trial, including through a natural bleaching event, with evidence for enhancing coral thermal resilience without repeated applications. Unlike traditional probiotics, which are selected for transient traits, MC10's efficacy stems from evolutionary compatibility with its host. By merging evolutionary biology with wildlife conservation, we establish a novel framework for climate-resilient restoration.

Results

Ruegeria population MC10 as a putative next-generation coral probiotic

To identify candidate probiotics, we cultured 419 *Ruegeria* isolates from five coral species (*Acropora* cf. *samoensis*, *Acropora solitaryensis*, *Favites pentagona*, *Platygyra acuta*, and *Oulastrea crispata*) across nine Hong Kong reef sites spanning diverse environmental gradients, including dissolved inorganic nitrogen concentrations of 21-107 µM (**Fig. S1**). Most bacterial isolates were obtained from coral mucus and skeleton (**Fig. 1A**).

Using PopCOGenT to delineate bacterial populations based on recombination barriers [23], we resolved 116 genetically cohesive *Ruegeria* populations, 29 of which included ≥3 isolates (**Fig. 1A**). Phylogenomic analysis confirmed monophyly for most populations, validating PopCOGenT's accuracy and enabling targeted surveys of probiotic dynamics. Among these, population MC10 emerged as a prime candidate, exhibiting genomic hallmarks of evolutionary young endosymbionts: expansion of IS (**Fig. 1B**) and proliferation of pseudogenes (**Fig. 1C**), as

quantified from closed genome assemblies (**Fig. S2**). Observed frameshift mutations disrupted key tricarboxylic acid (TCA) cycle genes, including aconitate hydratase (*ACO*), isocitrate dehydrogenase (*IDH1*), succinyl-CoA synthetase (*sucC*), malate dehydrogenase (*mdh*), and succinate dehydrogenase (*sdhA*) (**Fig. S3**). While 5′-terminal mutations likely abolished gene function, 3′-terminal disruptions may permit residual activity (**Fig. S3** & **Fig. S4**). Notably, pseudogenized loci lacked functional paralogs, suggesting heightened metabolic reliance on the coral host. Strikingly, only 8 of 15 closed MC10 genomes retained a complete TCA cycle, with similar heterogeneity observed in glycolysis, amino acid biosynthesis, and vitamin synthesis pathways (**Fig. S5**). While population MC10 exemplifies the hallmark features for next-generation probiotics, within population functional heterogeneity presents a potential source of variation in the efficacy of probiotic candidate lineages.

Host dependency may come at the cost of free-living capacity. To test this, we compared the growth patterns of the strain MC10-B4 (from MC10) in culture to its closely related *Ruegeria* strain MC15-BG7 (from MC15) sourced from the same *O. crispata* colony and a free-living fast-growing model strain *Ruegeria pomeroyi* DSS-3 isolated from coastal water [24] (**Fig. S6**). Consistent with its host-adapted genomic architecture, MC10-B4 exhibited reduced maximum growth rates (μ_{max} = 0.15 h^{-1}) in culture compared to its congenic relative MC15-BG7 (μ_{max} = 0.27 h^{-1}), but comparable to DSS-3 (μ_{max} = 0.12 h^{-1}). However, MC10-B4 achieved a 1.4-fold higher stationary-phase biomass yield (7.4 × 10□ CFU/mL) than MC15-BG7 (5.2 × 10□ CFU/mL) and a 7.7-fold increase over the DSS-3 (9.6 × 10□ CFU/mL) despite similar optical densities.

Phylogenetically, MC10's closest relative, MC46 (**Fig. 1A**), shares 99.45% 16S rRNA identity though diverges more genomically (average nucleotide identity or ANI: 86.88 ±

0.04%; **Fig. S7**), lacking IS expansion or pseudogenization (**Fig. 1B & 1C**). This contrast underscores MC10's recent origin.

Ruegeria MC10 is ubiquitously associated with diverse corals

To assess *Ruegeria* MC10's ecological distribution and coral host associations, we performed amplicon sequencing of two population-resolving marker genes, *parC* (encoding a topoisomerase IV subunit) and *ATP5B* (encoding an ATP synthase subunit). These markers were selected from over 700 conserved single-copy *Ruegeria* gene families and validated for their ability to resolve PopCOGenT-defined populations into monophyletic groups (**Fig. S8**). Custom primers achieved high recovery rates (64.8% for *parC*; 74.3% for *ATP5B*; **Table S1**), enabling targeted surveys across corals.

Phylogenetic analysis of amplicon sequences revealed MC10's presence in the five surveyed coral species (*O. crispata*, *Acropora solitaryensis*, *Plesiastrea* sp., *Porites* sp., and *P. acuta*) sampled from multiple reef sites in Hong Kong, as well as in seawater and sediment (**Fig. 2**). MC10's occurrence varied among individuals of the same coral species, typically constituting <2% of *Ruegeria* communities, where detected. Notably, *parC*- and *ATP5B*-based abundance estimates showed no linear correlation, particularly at proportions below 0.5% (**Fig. S9**), likely reflecting methodological challenges in quantifying low-abundance populations.

Attempts to quantify MC10's absolute abundance via qPCR were unsuccessful (**Fig. S10**), though its recurrent detection across phylogenetically distant corals and abiotic habitats underscores its ecological versatility. Despite methodological limitations for quantification, MC10's broad distribution and transient environmental persistence support its classification as a low-risk probiotic candidate, minimizing concerns of ecological disruption in restoration efforts.

Ruegeria MC10 increases thermal tolerance of a model cnidarian

To validate MC10's probiotic potential, we employed short-term acute thermal assays using the Coral Bleaching Automated Stress System (CBASS) [25,26], exposing Aiptasia (sensu *Exaipatasia diaphna*, a sea anemone model for coral-algal symbiosis) [27] to MC10-B4 at a pre-screened concentration of 10^6 CFU/ml (**Fig. S11**) with subsequent subjection to thermal stress (**Fig. S12**). The CBASS trial involved a 3-hour temperature ramp from 30 °C to target levels (34 °C, 36 °C, or 39 °C) under light, followed by a 3-hour hold, a 1-hour ramp-down to 30 °C in darkness, and an 11-hour overnight reprieve period [28]. Dark-acclimated photosynthetic efficiency (F_v/F_m) was used to measure response to heat stress at two time points: T7 (7th hour, immediately post-stress) to assess acute thermal tolerance, and T18 (18th hour, post-recovery) to evaluate thermal recovery [28]. Thermal tolerance was quantified by means of the Effective Dose 50 (ED50), which signifies the temperature at which a 50% decline in F_v/F_m relative to unstressed controls is observed [29]. MC10-inoculated Aiptasia exhibited significantly higher ED50 standardized thermal tolerance values than controls: a 1 °C increase at T7 ($P = 0.001$, one-way ANOVA) and a 0.6 °C increase at T18 ($P = 0.013$), demonstrating enhanced acute stress tolerance and recovery capacity, respectively (**Fig. 3A & 3B, Table S2**). Fluorescence in situ hybridization (FISH) assays with an MC10-specific probe confirmed presence of MC10-B4 in inoculated Aiptasia tissues (**Fig. S13**). These results, generated using Aiptasia's conserved symbiosis mechanisms, validate MC10's cross-host efficacy.

Real-world application demonstrates MC10's probiotic capacity through sustained thermal tolerance in reef corals

To evaluate *Ruegeria* MC10's probiotic capacity in natural settings, we conducted an 8-month field trial with *Acropora pruinosa*, initially designed to compare two evolutionarily distinct strains: MC10-B4 (exhibiting host-adapted genomic signatures) and its phylogenetically closely related (**Fig. 1**) congener MC15-BG7 (lacking such signatures), both isolated from the same *O. crispata* colony. Following a one-month laboratory inoculation regime (January 2024, **t0**) using genetically identical coral fragments to control for host variability, amplicon sequencing revealed stark contrasts in colonization success. MC10-B4 achieved significantly higher abundance in inoculated corals (median 18.331% of *Ruegeria* communities; 0.003% of total bacteria), significantly higher than the placebo group treated with autoclaved 0.22 μm-filtered seawater (AFSW) (2.410% of *Ruegeria* communities and < 0.001% of total bacteria; $P = 0.002$ and $P = 0.007$, respectively; one-way ANOVA; **Fig. 3C, Table S3**). In contrast, corals inoculated with MC15-BG7 showed no significant increase in MC15 abundance (median 3.242% of *Ruegeria* communities and < 0.001% of total bacteria) compared to the placebo group (0.251% of *Ruegeria* communities and < 0.001% of total bacteria; $P = 0.136$ and 0.281, respectively; one-way ANOVA; **Fig. 3D, Table S3**). Given MC10-B4's superior colonization, we prioritized these colonies for outplanting to Knob Reef, Hong Kong (**Fig. S14**).

The outplanted corals were monitored through September 2024 without further probiotic intervention, with measurements taken at five intervals: **t1** (1 month post-outplanting, February), **t3** (April), **t6** (July), **t7** (August), and **t8** (September). Crucially, a natural thermal stress event occurred during **t6-t8**, with degree heating weeks (DHW) reaching 8.8-18 ºC-weeks (**Fig. S15**), surpassing the 4-8 DHW bleaching threshold. During the 8-month monitoring period, MC10-B4-inoculated corals exhibited enhanced thermal tolerance, with significantly reduced signs of visual bleaching at the peak of thermal stress relative to placebo-treated group (CoralWatch Coral

Health Chart coloration scores at **t6**: 3.3 ± 0.5 vs. 2.5 ± 0.4, $P < 0.001$; **t7**: 3.2 ± 0.4 vs. 2.4 ± 0.4, $P < 0.001$; two-way ANOVA, Tukey's HSD; **Fig. 3E, Table S4**). Although partial recovery occurred in the subsequent month, neither MC10-B4-inoculated corals nor placebo-treated group fully regained pre-bleaching coloration levels by **t8**, indicating lingering stress effects. Concurrently, F_v/F_m' from corals inoculated with MC10-B4 varied by treatment at some timepoints ($P < 0.001$, two-way ANOVA, interaction effect), with probiotic-inoculated corals maintaining significantly higher F_v/F_m' (**t6**: $P < 0.001$; **t7**: $P < 0.001$; two-way ANOVA, Tukey's HSD; **Fig. 3F, Table S5**).

FISH assays with an MC10-specific probe at **t0**, **t6**, and **t8** confirmed MC10's persistent presence in coral tissues, particularly the gastrodermis (**Fig. 3G**), with additional nonsense probe confirming the hybridization specificity (**Fig. 3G**). MC10's low abundance within *Ruegeria* communities (median 0.23%) and *Ruegeria*'s minor representation in coral microbiomes (median 0.02%) precluded precise quantification, falling below the 0.5% detection threshold for reliable amplicon sequence variant (ASV) resolution [30].

To monitor microbiome dynamics in outplanted corals, we performed *parC* and 16S rRNA gene amplicon sequencing at **t0, t1, t3, t6**, and **t8** (**t7** samples unavailable; see Supplemental Methods). Analysis of β-diversity (compositional differences between communities) revealed that bacterial communities in MC10-B4-inoculated corals became progressively more similar to those in placebo-treated corals over time (**Fig. 4A**; ANOSIM). This convergence may reflect environmental acclimatization or dispersal-based homogenization at the reef site (groups separated by only 40 meters). Despite this community-level similarity, differential enrichment of individual taxa could be observed. During the bleaching phase (**t6**), ASV-based genus-level analysis identified significant enrichment of several Bacteroidetes genera (*Cryomorpha*,

Spirosoma, *Sunxiuqinia*, *Gelatiniphilus*, *Aureispira*, and *Flavobacterium*) and depletion of multiple Roseobacteraceae genera (*Sulfitobacter*, *Roseovarius*, *Pseudoruegeria*, *Donghicola*, *Aliiroseovarius*, and *Phaeobacter*) in MC10-B4-inoculated corals compared to the placebo group (FDR-corrected $P < 0.05$; ANCOM-BC; **Fig. 4B**). Crucially, most of these taxa showed no enrichment or depletion during pre-bleaching (**t3**) or post-bleaching (**t8**) phases (**Fig. S16**), indicating that MC10 may exert a microbial community restructuring effect during natural thermal stress.

This trial demonstrates that nursery-integrated probiotic treatments can enhance coral resilience to thermal stress without the need for post-outplanting intervention. MC10's sustained retention, even at low abundances, aligns with its evolutionary adaptations (e.g., pseudogenization-driven host dependency) and underscores its potential as a scalable tool for reef restoration.

Discussion

The accelerating loss of biodiversity under climate change demands innovative, scalable strategies to enhance resilience across ecosystems. While probiotics have shown promise in diverse systems, their scalability is universally hampered by transient colonization. Here, we advance a generalizable solution: probiotics selected through evolutionary principles, targeting bacteria undergoing irreversible transitions from free-living to host-dependent symbiosis. Using coral reefs as a model system, we identify *Ruegeria* MC10, a coral-associated bacterial population exhibiting genomic hallmarks of host adaptation. An 8-month field trial demonstrates that nursery MC10 inoculations confer sustained retention in corals through a natural bleaching event, enhancing thermal resilience without the need for repeated applications.

Evolutionary innovation in probiotic selection

Traditional probiotics, for marine organisms like corals, farmed shellfish and fish, as well as for terrestrial wildlife and farmed species, are selected for transient functional traits such as pathogen inhibition or nutrient provisioning [7,12,31]. However, uncertainty regarding their ability to persist long-term in host tissues limits scalability [32]. Here, we introduce a lifestyle-oriented strategy rooted in evolutionary genomics, shifting the paradigm from what probiotics do to how they evolve, their evolutionary trajectory. By targeting bacterial symbionts undergoing irreversible transitions from free-living to host-dependent symbiosis, we exploit their inherent genomic trajectory toward stable host association, arguably due to their putatively important role in holobiont functioning. This adaptation comes at a measurable and detectable genomic cost, as relaxed purifying selection during the lifestyle transition leads to the accumulation of mildly deleterious mutations, including IS expansion and pseudogene proliferation [33]. These genomic hallmarks, such as frameshift mutations or IS-driven disruptions in essential genes, reflect reduced selective pressure to maintain free-living metabolic pathways, driving metabolic dependency that favors sustained colonization [33,34]. *Ruegeria* MC10 exemplifies this novel approach, exhibiting hallmark signatures of these evolutionary young endosymbionts.

This strategy draws parallels with terrestrial, recently host-dependent endosymbionts, such as *Sodalis glossinidius* in Tsetse flies, which exhibit similar genomic erosion during early host adaptation [33,35]. However, while such terrestrial symbionts have not yet been repurposed as probiotics for economically or ecologically critical animals, our work demonstrates the feasibility of this approach in marine systems. MC10's success, retained in coral tissues for eight months post-inoculation, validates evolutionary principles as a blueprint for probiotic design

across ecosystems. For example, host-dependent symbionts in oysters, shrimp, or even terrestrial livestock could be harnessed to improve stress tolerance. Notably, at current we are naïve regarding the measurable host beneficial effect of such isolates. However, given our success here, one may argue it might be a rather more frequent than less occurrence.

By prioritizing evolutionary compatibility over inferred functional trait screening, this framework addresses a universal limitation in microbiome engineering: the uncertainty regarding long-term establishment of administered microbes [10]. For corals, MC10's persistence reduces reliance on repeated applications, a critical advance for large-scale restoration. Beyond reefs, this approach could transform probiotics for aquaculture, agriculture, and wildlife restoration, where long-term microbial colonization is essential. The integration of evolutionary theory into conservation practice marks a paradigm shift, enabling scalable solutions that align microbial interventions with natural host-microbe coevolution.

Facultative endosymbionts as host-generalist probiotics

Ruegeria MC10 is a facultative endosymbiont, given its variable presence among colonies of the same coral species and its presence as a free-living bacterium. Facultative endosymbionts undergoing early host adaptation exhibit a unique duality: genomic erosion drives metabolic dependency while retaining ecological flexibility. This paradox enables broad host ranges, as exemplified by *Sodalis glossinidius*, which persists in Tsetse flies and other insects despite pseudogenized metabolic pathways. Similarly, *Ruegeria* MC10's host versatility may arise from mechanisms that exploit universal host interfaces rather than lineage-specific adaptations. For instance, MC10's pseudogenized TCA cycle necessitates scavenging host-derived metabolites such as succinate or fumarate, pathways conserved across eukaryotes. This strategy aligns with

resource tracking, a mechanism highlighted by Lajoie and Parfrey [36], where microbial symbionts exploit conserved host resources (e.g., vitamins, metabolites) to colonize diverse hosts without specialization.

MC10's colonization of the coral gastrodermis likely involves eukaryotic receptors (e.g., integrins or lectins), akin to *Lactiplantibacillus plantarum*, which colonizes *Drosophila* spp. and many metazoan species through a conserved colonization island, allowing for wide use as a human and animal probiotic [37]. This touches upon the concept of ecological fitting, where pre-existing traits enable symbionts to colonize novel hosts without coevolution, a key driver of host generalization in facultative symbioses [36]. Additionally, MC10's transient environmental persistence reflects weak host selection pressures. As emphasized by Lajoie and Parfrey [36], when hosts impose minimal filtering (e.g., due to low symbiont abundance or metabolic byproduct exploitation), symbionts face reduced costs of adaptation to novel hosts, enabling niche expansion. MC10's low relative abundance in coral microbiomes likely minimizes its burden on the host, weakening selection for specialization and favoring persistence across taxa.

Scalability hinges on this generality. Unlike obligate symbionts restricted to single hosts [35], facultative endosymbionts like MC10 exploit conserved molecular gateways, enabling nursery-based priming of diverse corals.

Ruegeria MC10's growth dynamics balance evolutionary trade-offs and scalable cultivation

Growth rate comparisons between MC10 and its conventional *Ruegeria* relatives reveal that MC10 exhibits reduced proliferation under laboratory conditions, a phenotype potentially linked to pseudogenization of core metabolic pathways. Disrupted TCA cycle genes likely constrain ATP synthesis, while pseudogenized amino acid biosynthesis pathways increase reliance on

externally sourced metabolites, collectively imposing energetic inefficiencies. Despite these constraints, MC10 retains culturability, potentially through mechanisms like ribosomal frameshifting, where translational machinery bypasses frameshift mutations, or codon reassignment, where alternative decoding of stop codons permits partial gene expression. For example, *Salmonella enterica* translates 101 of 106 pseudogenes [38], though the specific mechanisms driving translation in this species remain untested. In general, such processes often produce misfolded proteins due to destabilized residue interactions, leading to energetic frustration and reduced functionality [39]. These molecular inefficiencies compound physiological deficits (e.g., reduced ATP yield), creating a dual burden that favors MC10's dependency on coral hosts for metabolic compensation. Thus, while pseudogene translation may sustain transient culturability, its inherent instability reinforces evolutionary trajectories toward host reliance, aligning genomic decay with ecological dependency.

Critically, MC10-B4's growth kinetics ($\mu_{max} = 0.15\ h^{-1}$) align with established probiotics like *Lactobacillus* spp. (0.12-0.23 h^{-1}) and marine probiotic *Phaeobacter inhibens* (0.16-0.18 h^{-1}). Its stationary-phase yields (7.4×10^8 CFU/mL) approach commercial probiotic standards (typically $\geq 10^9$ CFU/dose in pharmaceutical applications [40]), validating scalability for reef restoration. Industrial fermentation processes achieve high yields through tailored nutrient-rich media (e.g., whey-supplemented formulations for *Lactobacillus*) and advanced fermentation engineering (e.g., fed-batch cultivation), overcoming growth limitations in fastidious strains [41]. For MC10-B4, tailored media formulations, such as coral-mimicking nutrient formulations, could further enhance biomass production by compensating for pseudogenization-driven metabolic deficiencies. This industrial compatibility, coupled with MC10-B4's high cell density output, positions it as a tractable candidate for large-scale marine probiotic production.

Functional mechanisms underlying Ruegeria MC10's probiotic potential

Ruegeria MC10's benefits under thermal stress, including reduced algal photosynthetic efficiency loss and reduced bleaching, align with functional roles observed in terrestrial facultative endosymbionts. For example, *Serratia symbiotica* buffers its insect hosts against heat stress via metabolite exchange [42]. Though MC10's exact mechanisms remain unresolved, its retention in gastrodermis and pseudogenized metabolism suggest analogous stress-mitigating roles, potentially through stabilizing algal symbiont function or modulating host physiology. A key debate centers on whether probiotics act as nutritional sources to support host energetic demands [7,32,43]. MC10's sustained colonization over eight months argues against digestion-driven benefits, as degraded cells would not persist. Future experiments may leverage transcriptomics and metabolomics to resolve whether MC10 stabilizes algal symbiont function, modulates host immunity, or facilitates metabolite exchange during thermal stress.

Ecological flexibility and safety

While *Ruegeria* MC10's ecological flexibility, demonstrated by its cross-species efficacy in enhancing thermal tolerance across Hexacorallia, could raise concerns about off-target colonization, multiple factors converge that argue for its ecological safety. MC10 occurs naturally at low abundances (0.5-2% of coral-associated *Ruegeria* communities, where detected) across diverse hosts including faviid (*O. crispata*), acroporid (*A. solitaryensis*), and poritid (*Porites* sp.) corals, as well as in seawater and sediment. This widespread but sparse distribution may be due to constrained environmental fitness resulting from pseudogenized metabolism (e.g., disrupted TCA cycle), which may intrinsically limit competitive proliferation outside host tissues.

Our results also suggest that microbiome restructuring is fine-scale and restricted to context-specific depletion of certain Roseobacteraceae genera during bleaching events, which may suggest transient niche restructuring. Of note, MC10's evolutionary trajectory toward host dependency manifests in tissue-specific localization (gastrodermis) and ultralow persistence abundances (<0.005% total bacteria), reducing environmental shedding risks. Collectively, these attributes, encompassing natural rarity, self-limiting metabolic constraints, and host-adapted persistence, position MC10 as a coral “core microbiome” taxon: a low-abundance generalist that supports holobiont function without ex hospite ecosystem disruption, making it ideally suited for multi-species restoration [44].

Microbiome dynamics and stability

A central goal of probiotic intervention aligns with microbial transfer therapy (MTT) in human medical settings: introducing beneficial taxa to reset dysbiotic microbiomes and enhance resilience through community restructuring [21,45]. By leveraging *Ruegeria* MC10 as a keystone inoculant, we achieved microbiome modulation without long-term destabilization, evidenced by transient β-diversity differences that peaked post-inoculation but converged with placebo-treated corals over eight months. Crucially, during bleaching stress (**t6**), MC10-B4 association co-occurred with a functional microbiome reset characterized by enrichment of Bacteroidetes polysaccharide degradation specialists (*Cryomorpha*, *Spirosoma*, *Sunxiuqinia*, *Gelatiniphilus*, *Aureispira*, *Flavobacterium*) [46], ROS-tolerant taxa (*Deinococcus*, *Desertibacter*, *Spirosoma*) [47], *Vibrio*-predatory specialist (*Aureispira*) [48], and known probiotics (*Veillonella*, *Limosilactobacillus*) [49,50]. Concurrent depletion of multiple Roseobacteraceae genera (*Sulfitobacter*, *Roseovarius*, *Pseudoruegeria*, *Donghicola*, *Aliiroseovarius*, *Phaeobacter*)

indicated competitive restructuring within this ecologically critical family, notably including both MC10 itself and the established probiotics *Phaeobacter* [51]). This taxonomic shift, largely absent pre- or post-bleaching, argues MC10 primed the microbiome for stress-responsive optimization.

While algal symbiont switching (e.g., from sensitive *Cladocopium* to resilient *Durusdinium*) represents a known thermal adaptation pathway in some corals [52], this mechanism is unlikely to explain our results. Regional characterization confirms Hong Kong corals, including *Acropora* spp., exclusively harbor *Cladocopium* spp. (except *O. crispata*) [53]. This consistency across host genotypes and sites makes algal symbiont-mediated thermal tolerance gains as an alternative explanation for MC10-B4's efficacy in inoculated *A. pruinosa* unlikely.

Next-generation probiotics as a scalable solution

Coral restoration typically involves growing colony fragments in nurseries before outplanting [54,55], a framework highly compatible with integrating next-generation probiotics like Ruegeria MC10 as a nursery-applied treatment. Our 8-month field trial provides a proof of concept that evolutionarily selected probiotics can achieve sustained retention and enhance stress tolerance evading the need for post-outplanting intervention. However, critical knowledge gaps remain for evaluating long-term restoration success, an issue plaguing coral restoration initiatives globally [55]. Multi-year data on coral survival, reproductive fitness, and reef-scale outcomes (e.g., biodiversity recovery, structural complexity) are essential next steps to validate this approach.

Notwithstanding these open questions, the evolutionary design of next-generation probiotics offers a uniquely promising pathway for scalability: host dependency minimizes post-outplanting dispersal risks, while transient environmental viability and cross-species efficacy enable efficient

"probiotic priming" within existing nursery workflows. Given the accelerating pace of ocean warming, which increasingly outstrips natural adaptation [56], such scalable interventions represent one of the most viable options to bridge the resilience gap: the widening disparity between organismal stress tolerance and environmental insult magnitude that exceeds compensatory capacities of acclimation or adaptation.

By aligning mechanistic research with scalable delivery systems, the here-presented approach offers a blueprint to explore microbial-driven resilience across ecosystems. Further validation of long-term ecological impacts and industrial-scale production will be crucial, but the urgency of the coral crisis demands parallel exploration of multiple strategies, among which evolution-guided probiotics stand as a compelling, scalable candidate.

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Data and materials availability: Raw sequencing data are available on NCBI under several BioProjects with the private access link for reviewers. The raw reads of 409 and assembled genomes of 419 *Ruegeria* isolates (10 genomes missing raw data) sequenced on the second-generation platform (DNBseq) and 34 *Ruegeria* isolates sequenced on the third-generation platform (Nanopore) are available under NCBI BioProject accessions PRJNA1264799 (https://dataview.ncbi.nlm.nih.gov/object/PRJNA1264799?reviewer=mp7rulknapinfb95c9733nf72r) and PRJNA1275854 (https://dataview.ncbi.nlm.nih.gov/object/PRJNA1275854?reviewer=g4mffnb8b965tq6b2662cpt6i8), respectively. The *parC* and *ATP5B* amplicon sequencing data for natural coral samples are available under PRJNA1275610 (https://dataview.ncbi.nlm.nih.gov/object/PRJNA1275610?reviewer=nst2vifa08hqr8otcjvv39ge4

l) and PRJNA1275576 (https://dataview.ncbi.nlm.nih.gov/object/PRJNA1275576?reviewer=g865emc830spbkko9b86qtcc35), respectively. The 16S rRNA and *parC* amplicon sequencing data for outplanted corals are available under PRJNA1275585 (https://dataview.ncbi.nlm.nih.gov/object/PRJNA1275585?reviewer=9ne88342f7at8ndur1klq05mo8) and PRJNA1275617 (https://dataview.ncbi.nlm.nih.gov/object/PRJNA1275617?reviewer=e6g3aatk7pg8r6m6ao0riovjnu), respectively. All data will be publicly accessible upon publication. The scripts used for genome assembly and annotations, population genomic analysis, amplicon data analysis, and pseudogene analysis have been deposited in an online repository (https://github.com/444thLiao/CoralProbiotic).

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Figure Legends

Fig. 1 *Ruegeria* MC10's phylogenetic position and genomic signatures showing recent host dependency. (A) Rooted maximum-likelihood phylogenomic tree comprising 419 *Ruegeria* isolates associated with five coral species sampled from nine Hong Kong reef sites, along with 26 *Ruegeria* genomes retrieved from public databases. This tree was constructed with IQ-TREE based on concatenated single-copy orthologous genes at the amino acid level. PopCOGenT was used to delineate the *Ruegeria* populations (i.e., main clusters or MCs). The 19 MCs that contain representative members additionally sequenced by Nanopore were labelled with numbers in the tree. From the inner to outer rings: (1) denotes 29 *Ruegeria* populations which consists of at least three isolates as defined by PopCOGenT, along with MC46, the sister group of MC10; (2) - (5) denote the source of *Ruegeria* isolates with each corresponding to coral species, sampling site, coral compartment, and coral colony replicate, respectively. (**B)** Stacked bars showing the number of IS and IS family composition in 34 newly closed *Ruegeria* genomes and the reference genome *Ruegeria pomeroyi* DSS-3. (**C)** Number of pseudogenes in 34 newly closed *Ruegeria* genomes and the reference genome *R. pomeroyi* DSS-3. Pseudogenes were grouped by the mechanism of formation, namely "IS disruption", "Frameshift mutations", "Nonsense mutations", and "Singletons". "Unclassified" pseudogenes are those that cannot be assigned to any of the three defined mechanisms.

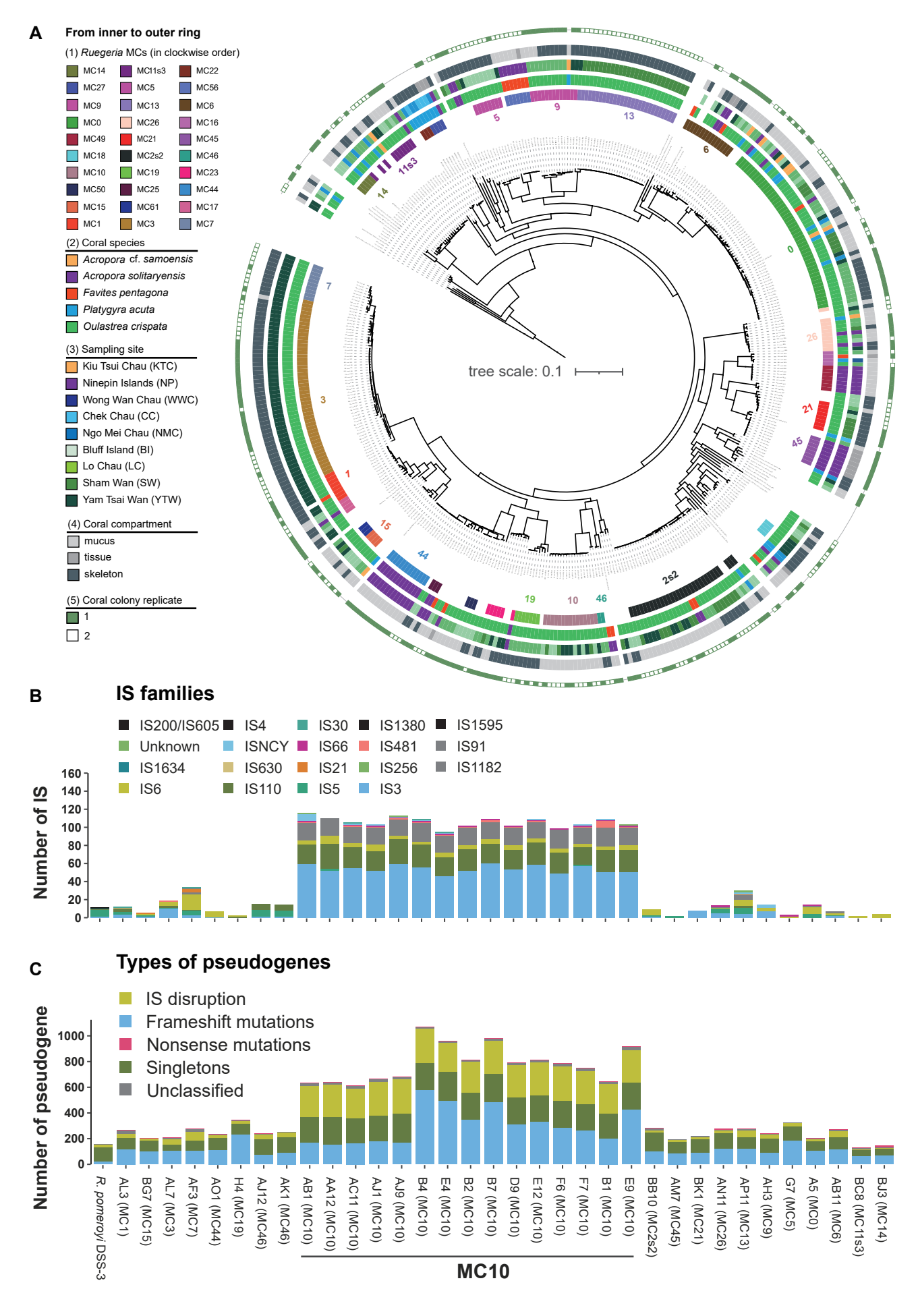
Fig. 2 Relative abundance of *Ruegeria* MC10 in coral holobionts and environmental samples from Hong Kong reefs. (**A)** The relative abundance of MC10 in *Ruegeria* communities was quantified based on population-resolving *ATP5B* and *parC* amplicon sequencing across natural reefs in Hong Kong. Different bar plots under the same coral genus represented for different coral colonies (i.e., 15 colonies for *Oulastrea* sp., 11 for *Platygyra* sp., 10 colonies for

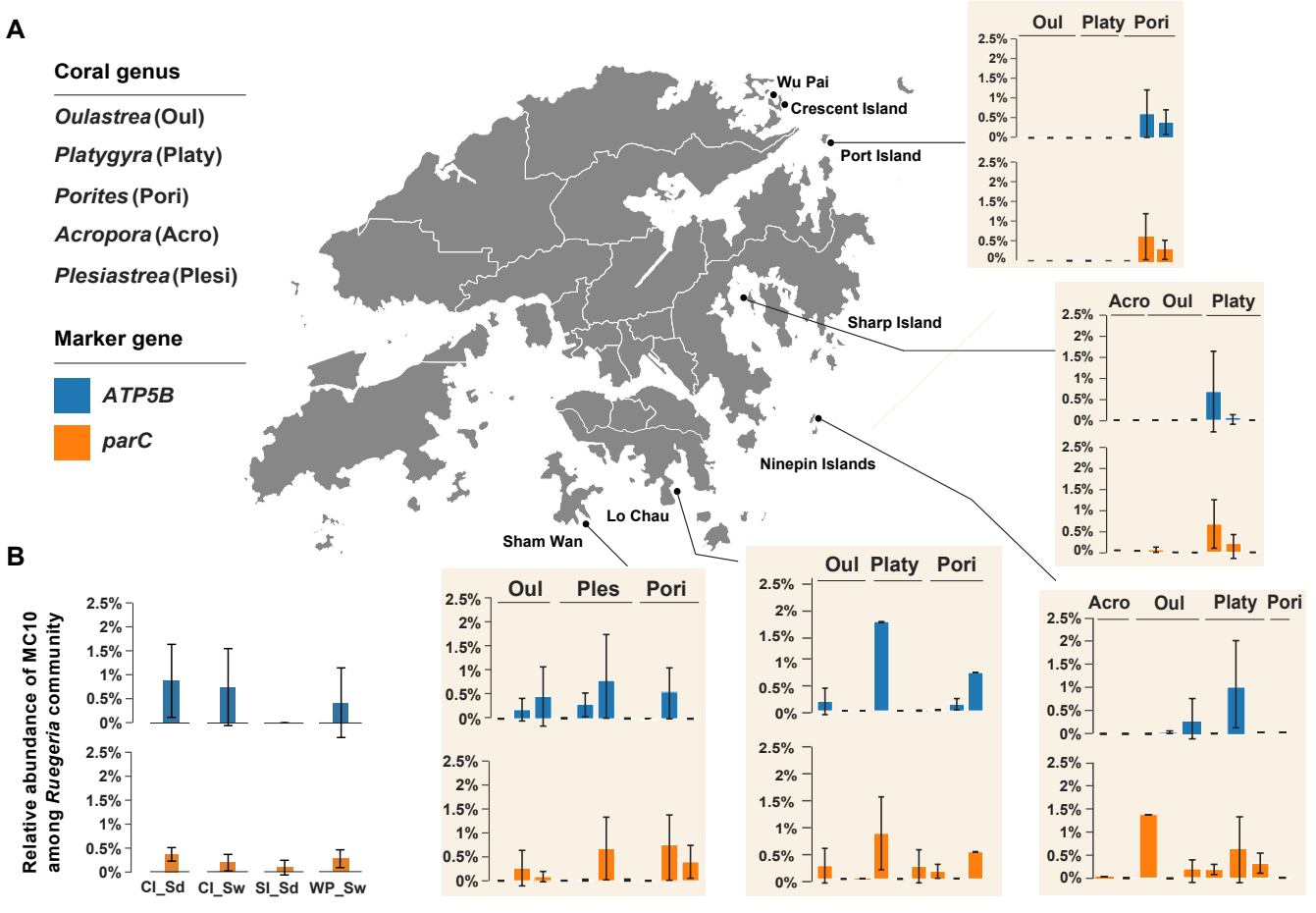
Porites sp., 4 colonies for *Acropora* sp., and 4 colonies for *Plesiastrea* sp. across five sites). Each bar plot was displayed with the mean value and standard deviation (SD) calculated from three compartments (i.e., mucus, tissue and skeleton) for the same coral colony. (**B)** The relative abundance of MC10 in seawater and sediment samples across different locations. The error bar denotes standard deviation among three replicates. CI, Crescent Island; SI, Sharp Island; WP, Wu Pai. Sd, sediment; Sw, seawater.

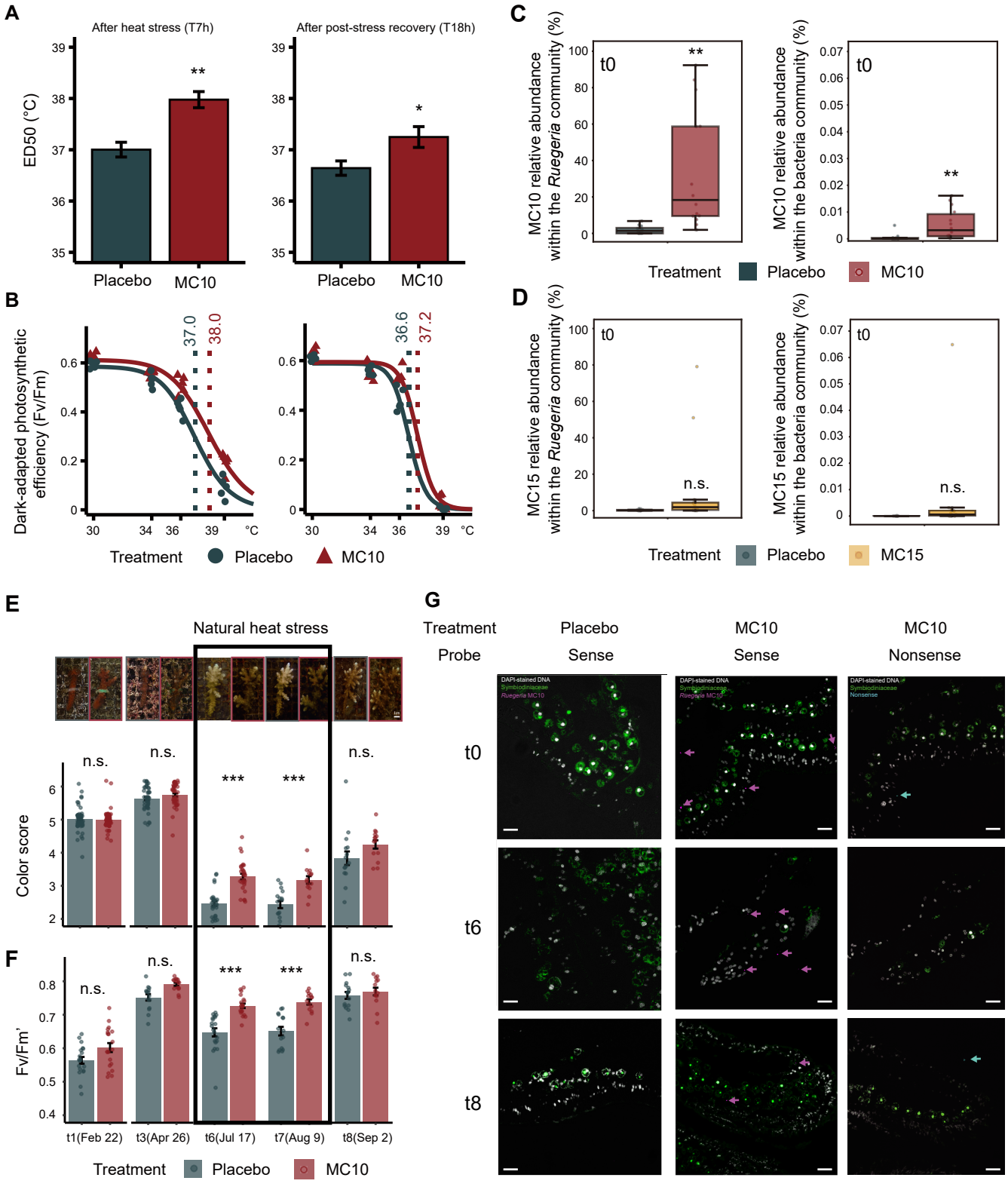
Fig. 3 Application of *Ruegeria* MC10-B4 through laboratory and field trials. (**A**) ED50 values of Aiptasia H2 inoculated with *Ruegeria* MC10-B4 or placebo FSW after heat stress (T7) and post-stress recovery (T18). Data were presented with the mean values and standard errors. (**B**) ED50 values modeled based on log-logistic regression curves of dark-adapted photosynthetic efficiency (F_v/F_m) across experimental temperatures. F_v/F_m was measured at 7 h and 18 h of the CBASS with one-hour dark acclimation. Treatment-specific ED50 values were denoted as vertical lines. The relative abundance of **(C)** MC10 and **(D)** MC15 in the *Ruegeria* (left axis; based on *parC* amplicon) and total bacteria community (right axis; combined 16S rRNA and *parC* gene amplicon) following twice-weekly inoculations over a month. The central line of each box indicates the median value with the upper and lower error bar showing maximum and minimum potential variabilities, respectively. **(E)** Coral phenotype and coloration, and **(F)** effective quantum yield (F_v/F_m') were assessed on MC10-B4-inoculated and placebo-treated corals across five timepoints: 1, 3, 6, 7 and 8 months post-outplanting (**t1**, **t3**, **t6**, **t7**, **t8**, respectively). Data were presented with the mean values and standard errors. **(G)** Localization of MC10 in coral *A. pruinosa* after one-month inoculations (**t0**, before outplanting), six months post-outplanting (**t6**, during bleaching), and eight months post-outplanting (**t8**, post-bleaching). Representative confocal images depicting the hybridization of high-resolution *parC* probe

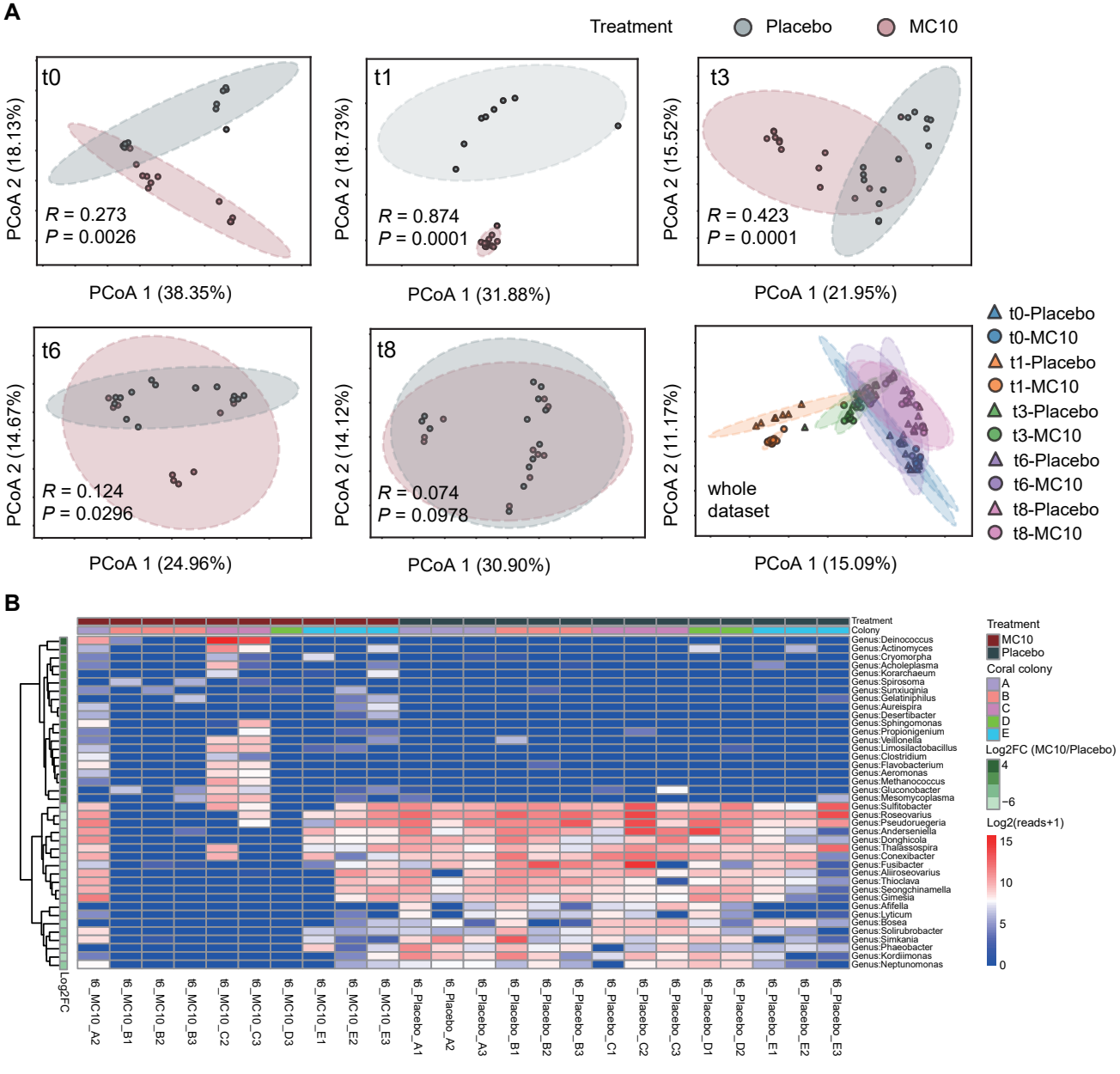
specifically targeting MC10 labeled with Cy3 (purple). The purple arrows pointed at the specific location of MC10 in the coral gastrodermis. The blue arrows pointed at the non-specific binding sites. Grey, DAPI-stained dsDNA; Green, autofluorescence of Symbiodiniaceae; Scale bars, 10 μm. All significant differences were indicated in bold and marked with asterisks (***: $P < 0.001$, **: $P < 0.01$; *: $P < 0.05$; n.s.: non-significant).

Fig. 4 Coral microbiome dynamics during field trial. (**A)** Principal Coordinates Analysis (PCoA) of coral holobiont-associated bacterial communities based on Bray-Curtis dissimilarity, comparing placebo and MC10-B4-treated groups across five timepoints: baseline (**t0**, pre-outplanting) and 1, 3, 6, and 8 months post-outplanting (**t1**, **t3**, **t6**, **t8**, respectively). Ellipses represent 95% confidence intervals for group centroids. ANOSIM was used to assess the separation of the placebo and MC10-B4-treated groups, with R values interpreted as: little to no ($R < 0.25$), weak ($0.25 \leq R < 0.5$), moderate ($0.5 \leq R < 0.75$), and strong ($R \geq 0.75$) separation. **(B)** Differentially abundant taxa between placebo and MC10-B4-treated corals at t6 (during bleaching) identified by ANCOM-BC analysis ($P < 0.05$, FDR corrected). A heatmap displaying top 20 significantly upregulated (positive log2FC) and downregulated (negative log2FC) genera, respectively. A log2-transformed read counts (log2[reads+1]) were shown to indicate the absolute fold-change magnitudes of taxa. Pseudocounts were added to handle the case of zeros in the calculation.


A
From inner to outer ring
(1) Ruegeria MCs (in clockwise order)
MC14
MC11s3
MC22
MC27
MC5
MC56
MC9
MC13
MC6
MC0
MC26
MC16
MC49
MC21
MC45
MC18
MC2s2
MC46
MC10
MC19
MC23
MC50
MC25
MC44
MC15
MC61
MC17
MC1
MC3
MC7
(2) Coral species
Acropora cf. samoensis
Acropora solitaryensis
Favites pentagona
Platygyra acuta
Oulastrea crispata
(3) Sampling site
Kiu Tsui Chau (KTC)
Ninepin Islands (NP)
Wong Wan Chau (WWC)
Chek Chau (CC)
Ngo Mei Chau (NMC)
Bluff Island (BI)
Lo Chau (LC)
Sham Wan (SW)
Yam Tsai Wan (YTW)
(4) Coral compartment
mucus
tissue
skeleton
(5) Coral colony replicate
1
2
tree scale: 0.1
B
IS families
IS200/IS605
IS4
IS30
IS1380
IS1595
Unknown
ISNCY
IS66
IS481
IS91
IS1634
IS630
IS21
IS256
IS1182
IS6
IS110
IS5
IS3
Number of IS
C
Types of pseudogenes
IS disruption
Frameshift mutations
Nonsense mutations
Singletons
Unclassified
Number of pseudogene
R. pomeroyi DSS-3
AL3 (MC1)
BG7 (MC15)
AL7 (MC3)
AF3 (MC7)
AO1 (MC44)
H4 (MC19)
AJ12 (MC46)
AK1 (MC46)
AB1 (MC10)
AA12 (MC10)
AC11 (MC10)
AJ1 (MC10)
AJ9 (MC10)
B4 (MC10)
E4 (MC10)
B2 (MC10)
B7 (MC10)
D9 (MC10)
E12 (MC10)
F6 (MC10)
F7 (MC10)
B1 (MC10)
E9 (MC10)
BB10 (MC2s2)
AM7 (MC45)
BK1 (MC21)
AN11 (MC26)
AP11 (MC13)
AH3 (MC9)
G7 (MC5)
A5 (MC0)
AB11 (MC6)
BC8 (MC11s3)
BJ3 (MC14)
MC10


A
Coral genus
Oulastrea (Oul)
Platygyra (Platy)
Porites (Pori)
Acropora (Acro)
Plesiastrea (Plesi)
Marker gene
ATP5B
parC
Wu Pai
Crescent Island
Port Island
Sharp Island
Ninepin Islands
Lo Chau
Sham Wan
Oul Platy Pori
Acro Oul Platy
Oul Ples Pori
Oul Platy Pori
Acro Oul Platy Pori
2.5%
2%
1.5%
1%
0.5%
0%
B
Relative abundance of MC10 among Ruegeria community
Cl_Sd
Cl_Sw
Sl_Sd
WP_Sw


A
After heat stress (T7h)
After post-stress recovery (T18h)
ED50 (°C)
Placebo
MC10
B
Dark-adapted photosynthetic efficiency (Fv/Fm)
37.0
38.0
36.6
37.2
°C
Treatment
Placebo
MC10
C
MC10 relative abundance within the Ruegeria community (%)
MC10 relative abundance within the bacteria community (%)
t0
Treatment
Placebo
MC10
D
MC15 relative abundance within the Ruegeria community (%)
MC15 relative abundance within the bacteria community (%)
n.s.
MC15
E
Natural heat stress
Color score
n.s.
***
F
Fv/Fm'
t1(Feb 22)
t3(Apr 26)
t6(Jul 17)
t7(Aug 9)
t8(Sep 2)
G
Treatment
Probe
Placebo
Sense
MC10
Sense
MC10
Nonsense
t0
t6
t8


A
Treatment
Placebo
MC10
t0
R = 0.273
P = 0.0026
PCoA 2 (18.13%)
PCoA 1 (38.35%)
t1
R = 0.874
P = 0.0001
PCoA 2 (18.73%)
PCoA 1 (31.88%)
t3
R = 0.423
P = 0.0001
PCoA 2 (15.52%)
PCoA 1 (21.95%)
t6
R = 0.124
P = 0.0296
PCoA 2 (14.67%)
PCoA 1 (24.96%)
t8
R = 0.074
P = 0.0978
PCoA 2 (14.12%)
PCoA 1 (30.90%)
whole dataset
PCoA 2 (11.17%)
PCoA 1 (15.09%)
t0-Placebo
t0-MC10
t1-Placebo
t1-MC10
t3-Placebo
t3-MC10
t6-Placebo
t6-MC10
t8-Placebo
t8-MC10
B
Treatment
Colony
Log2FC
Genus:Deinococcus
Genus:Actinomyces
Genus:Cryomorpha
Genus:Acholeplasma
Genus:Korarchaeum
Genus:Spirosoma
Genus:Sunxiuqinia
Genus:Gelatinphilus
Genus:Aureispira
Genus:Desertibacter
Genus:Sphingomonas
Genus:Propionigenium
Genus:Veillonella
Genus:Limosilactobacillus
Genus:Clostridium
Genus:Flavobacterium
Genus:Aeromonas
Genus:Methanococcus
Genus:Gluconobacter
Genus:Mesomycoplasma
Genus:Sulfitobacter
Genus:Roseovarius
Genus:Pseudoruegeria
Genus:Anderseniella
Genus:Donghicola
Genus:Thalassospira
Genus:Conexibacter
Genus:Fusibacter
Genus:Aliiroseovarius
Genus:Thioclava
Geongcheonamella
Genus:Gimesia
Genus:Afifella
Genus:Lyticum
Genus:Bosea
Genus:Solirubrobacter
Genus:Simkania
Genus:Phaeobacter
Genus:Kordiimonas
Genus:Neptunomonas
t6_MC10_A2
t6_MC10_B1
t6_MC10_B2
t6_MC10_B3
t6_MC10_C2
t6_MC10_C3
t6_MC10_D3
t6_MC10_E1
t6_MC10_E2
t6_MC10_E3
t6_Placebo_A1
t6_Placebo_A2
t6_Placebo_A3
t6_Placebo_B1
t6_Placebo_B2
t6_Placebo_B3
t6_Placebo_C1
t6_Placebo_C2
t6_Placebo_C3
t6_Placebo_D1
t6_Placebo_D2
t6_Placebo_E1
t6_Placebo_E2
t6_Placebo_E3
Treatment
MC10
Placebo
Coral colony
A
B
C
D
E
Log2FC (MC10/Placebo)
4
−6
Log2(reads+1)
15
10
5
0