Supplementary figures

Chapter 2: Engineering coral resilience: functional testing of probiotic bacteria to increase thermal stress tolerance of the coral model Aiptasia

Prepared for submission to *BMC Microbiome*, 2025

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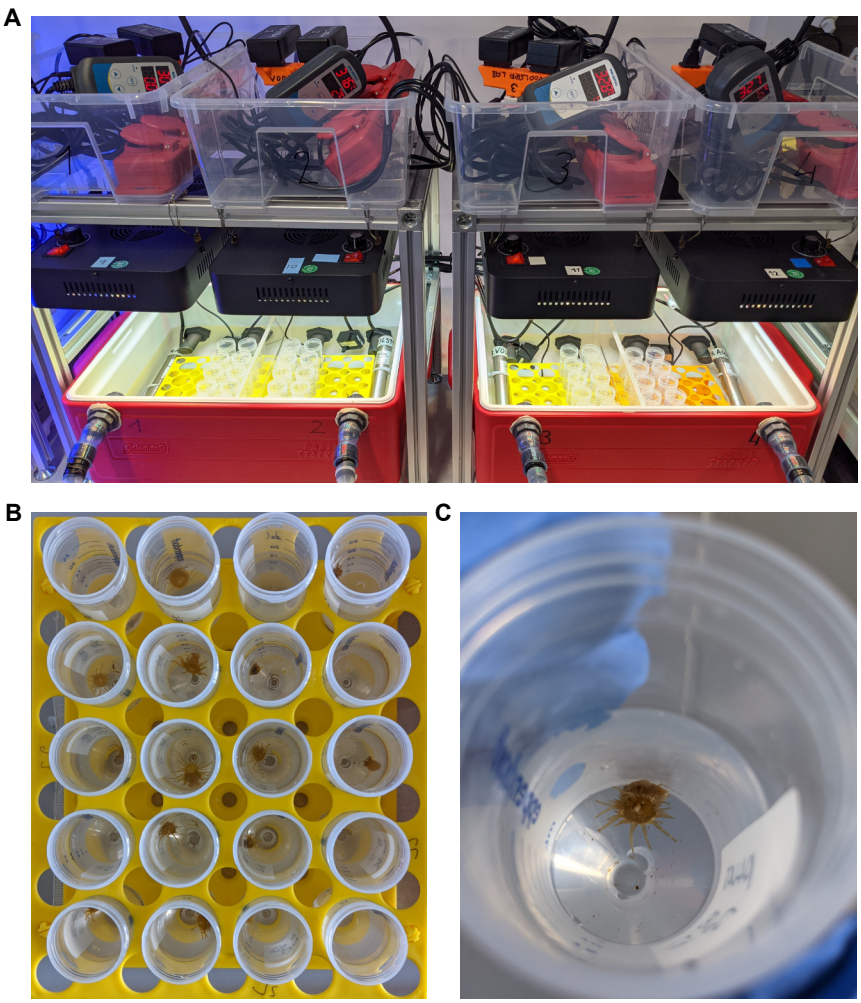

Figure S5. Coral Bleaching Automated Stress System (CBASS) setup for Aiptasia anemones. (A) The CBASS system consists of four temperature treatments, built into two tanks, separated by plexiglass. Each tank is equipped with a programmable thermostat (Inkbird ITC-310 T-B), two chillers, one heater, one temperature logger, and a dimmable 165 W full-spectrum LED aquarium light adjusted to Aiptasia culture conditions (~ 80 µmol photons m^{-2} s^{-1}). Aiptasia anemones are placed into each tank using a centrifuge tube rack and weights. **(B)** A centrifuge tube rack with 20 Aiptasia anemones settled in 25 mL centrifuge tubes filled with 20 mL of a bacterial inoculation solution or FASW. Centrifuge tube caps are removed prior to the CBASS run to ensure light accessibility. **(C)** Close-up of an Aiptasia anemone settled to the side of a 25 mL centrifuge tube. Photo credits: Melanie Dörr.

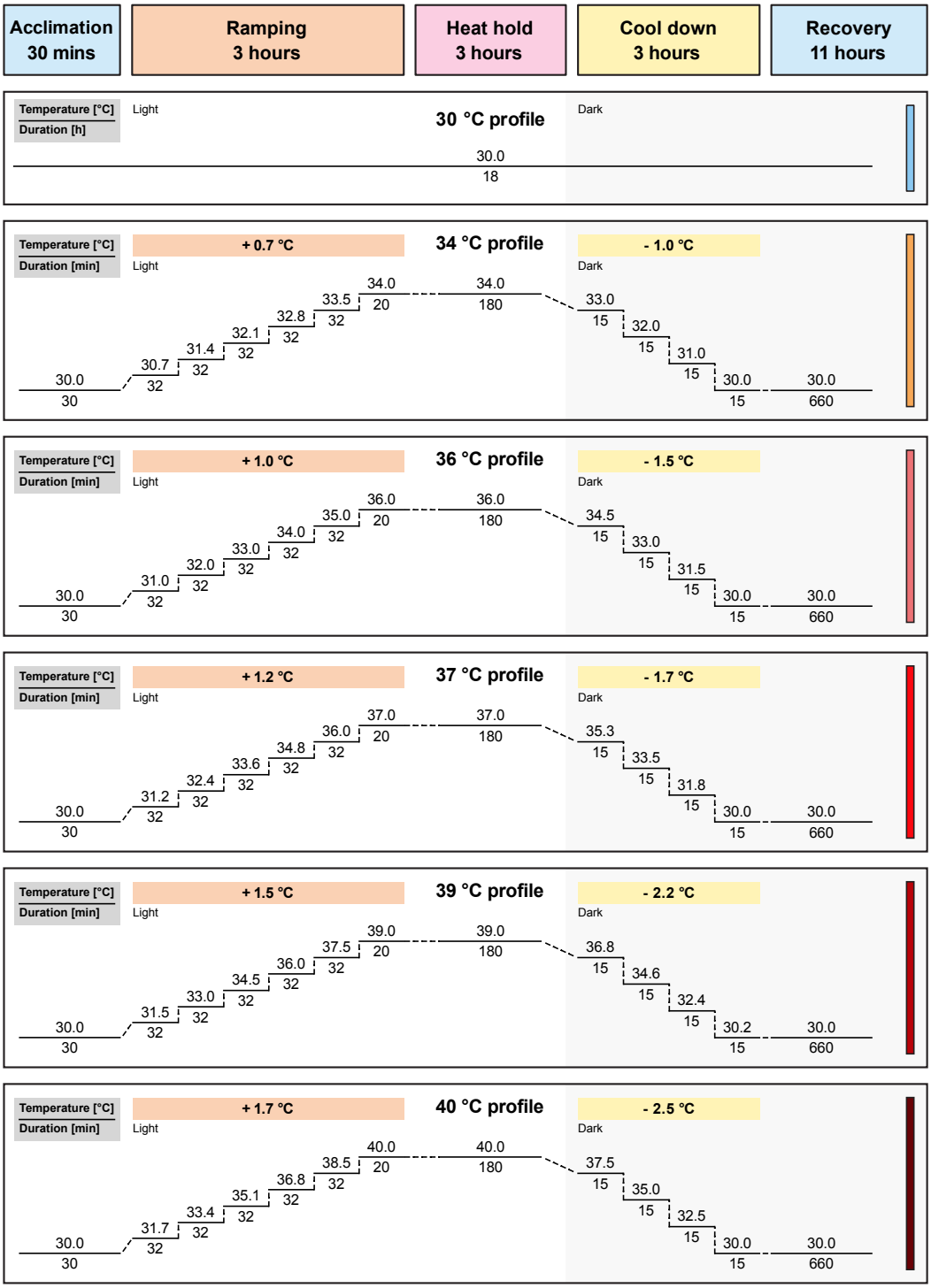

Figure S6. CBASS Inkbird ITC-310 T-B 12-step temperature profiles. Prior to heat stress, anemones are placed into their respective temperature treatment tanks pre-warmed to 30 °C to acclimate for 30 min. The baseline tank (30 °C profile) was maintained at 30 °C for the 18 h heat stress assay. The temperatures in the heat stress tanks were incrementally ramped up in six steps to 34 °C (baseline + 4 °C, medium), 36/37 °C (baseline + 6/7 °C, high), and 39/40 °C (baseline + 9/10 °C, extreme) over the course of 3 hours. The heat stress temperatures were held for 3 hours, and ramped down to 30 °C in four 15-minute steps while animals were dark-acclimated. The temperature was kept at 30 °C for the 11-hour (660 min) overnight recovery period. For each CBASS assay, either 30 °C, 34 °C, 36 °C, and 39 °C temperature profiles or 30 °C, 34 °C, 37 °C, and 40 °C temperature profiles were used.

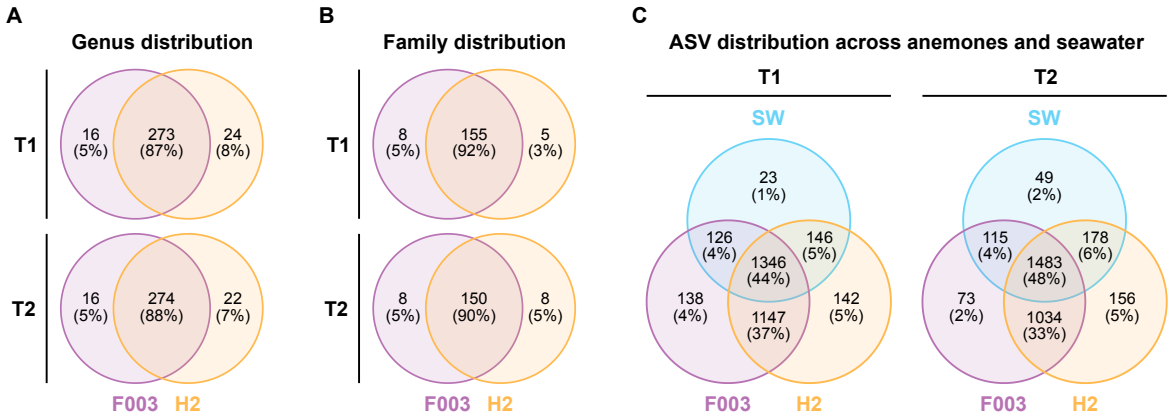

Figure S7. Comparison of bacterial genus, family, and ASV distribution across microbiomes of Aiptasia strains F003 and H2. Venn diagrams of **(A)** bacterial genus, **(B)** bacterial family, and **(C)** ASV distribution of F003 (purple, n = 10), H2 (orange, n = 10), and seawater (SW, blue, n = 2) over time point 1 (T1) and time point 2 (T2). The Venn overlap represents bacterial genera, families, or ASVs present across groups. Note that Aiptasia strains share around 90% of their observed bacterial genera and families. Around 45% of observed ASVs were shared between both anemone strains and the seawater in which they were reared.

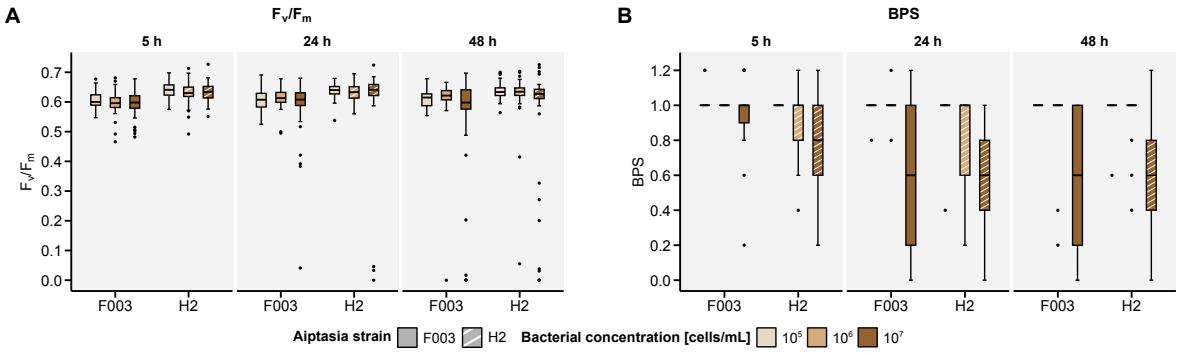

Figure S8. Effect of bacterial density on Aiptasia physiology after incubation with three bacterial concentrations over time. Aiptasia sea anemones of strain F003 and H2 (F003 = filled, H2 = striped) were inoculated with 10^5, 10^6, and 10^7 bacterial cells/mL to pre-screen bacterial isolates for beneficial potential. **(A)** F_v/F_m and **(B)** BPS measurements were taken after 5 h, 24 h, and 48 h of inoculation. F_v/F_m measures were generally less affected by bacterial density, with outliers representing exposures to potentially pathogenic bacteria. While not inherently observed for all bacterial isolates, exposure to 10^7 cells/mL tended to affect BPS scores of both anemone strains, with increasingly pronounced effects over time, whereas a concentration of 10^5 bacterial cells/mL had no effect. Thus, a concentration of 10^6 cells/mL and incubation of 24 h enabled the discrimination between detrimental and non-detrimental bacterial isolates, without potential density effects.

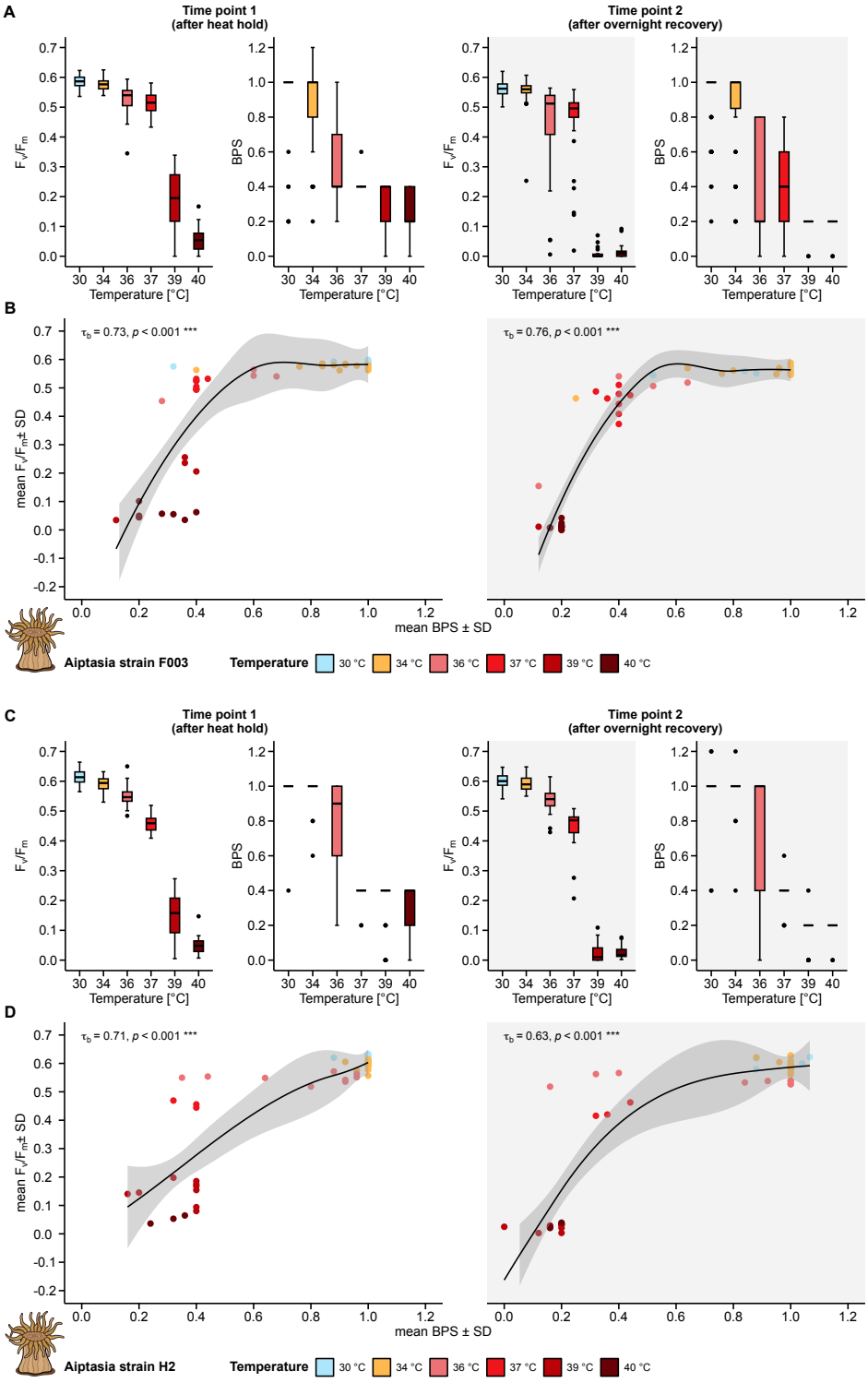

Figure S9. Relationship between F_v/F_m and BPS across CBASS stress temperatures of Aiptasia strain F003 and H2. (A, C) Spread of all measured dark acclimated F_v/F_m and BPS values across CBASS temperature treatments of (A) F003 (n = 468 values) and (C) H2 (n = 470 values) anemones (30 °C = light blue, 34 °C = yellow, 36 °C = light red, 37 °C = red, 39 °C = dark red, 40 °C = maroon) and measurement time points 1 and 2. Both F_v/F_m and BPS values drop with increasing heat stress temperatures. **(B, D)** Ordinal association between F_v/F_m and BPS ranks, with each point representing a pairwise comparison of ranked observations in (B) F003 and (D) H2 (mean of n = 5 animals per treatment and CBASS temperature). Only bacteria-inoculated or control anemones were considered, where both (F_vF_m and BPS) values were recorded (n = 12 treatments for both strains). Both time points showed a statistically significant, high positive correlation ($p < 0.001$) with higher ranks of F_v/F_m corresponding to higher ranks of BPS. The coefficient τ ranges from -1 (perfect discordance) to +1 (perfect concordance), and significance levels are $p \leq 0.001$: ***.