Coral probiotics promote tissue-specific and putatively beneficial microbiome restructuring in a coral dwelling fish

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INTRODUCTION

Background:

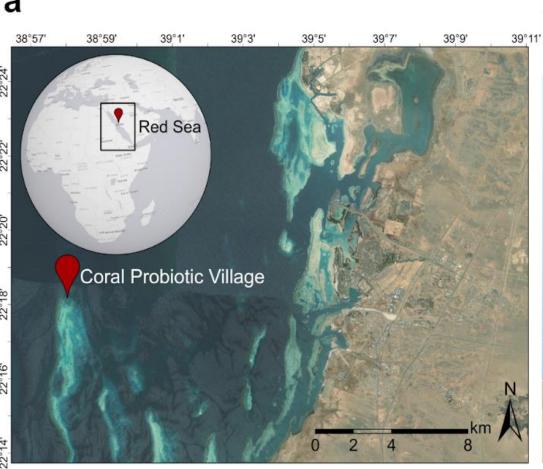
- Coral reefs are facing unprecedented threats (e.g., coral bleaching, diseases, and pollution).
- Beneficial Microorganisms for Corals (BMCs) are probiotics that enhance coral resilience¹.
- Effects on other associated reef organisms, like fish, remain unexplored².

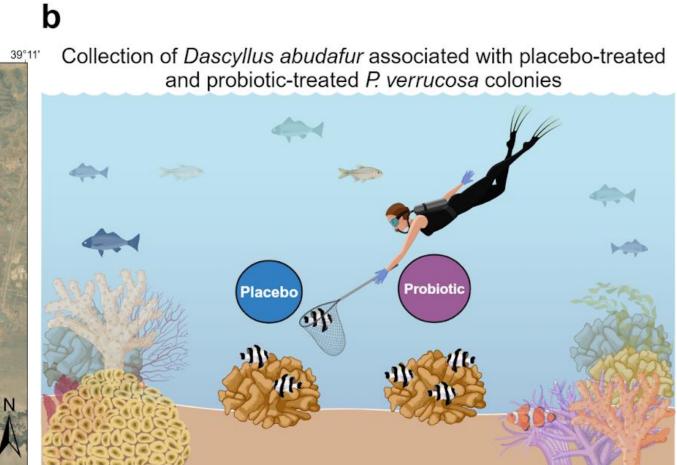
Objective:

 Assess how BMCs reshape the microbiome of a coral-associated fish: Dascyllus abudafur.

METHODS

L. Experimental Design Workflow





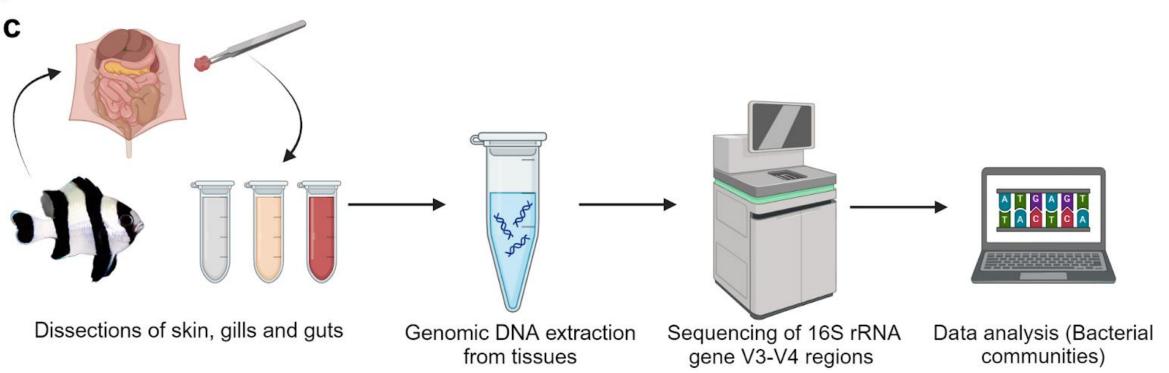


Fig. 1. a) Study location; **b)** *in situ* fish collection procedure; **c)** tissue dissection and bacterial community analysis via DNA extraction and NGS.

- a) BMCs applied to *Pocillopora verrucosa* corals in the Coral Probiotics Village, Al Fahal, Red Sea³.
- b) Sampled *D. abudafur* from placebo and probiotictreated *P. verrucosa* colonies.
- Dissection of skin, gills, and gut tissues followed by DNA extraction and 16S community analysis.

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- 2) Peixoto, R. S. et al. Harnessing the microbiome to prevent global biodiversity loss. *Nat Microbiol* 7, 1726–1735 (2022).
- 3) Delgadillo-Ordoñez, N. et al. Probiotics reshape the coral microbiome in situ without detectable off-target effects in the surrounding environment. *Commun Biol* 7, 434 (2024).

RESULT

2. Microbiome Shifts in Fish Tissues

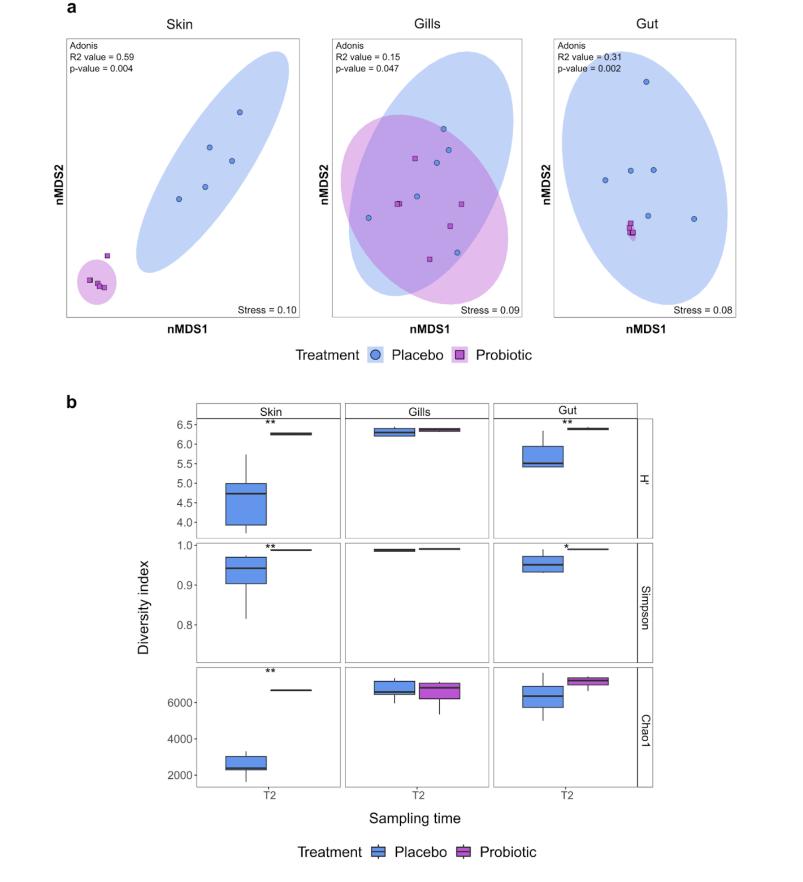


Fig. 2. Bacterial community shifts in *D. abudafur* by tissue type, treatment, and time (T2). **a)** nMDS ordination by treatment. **b)** Alpha diversity metrics. Statistical significance is denoted with * (**, ***).

- Significant shifts in skin and gut microbiomes post-probiotic treatment.
- Higher alpha diversity in probiotic-treated skin and gut.

4. Differentially Abundant ASVs

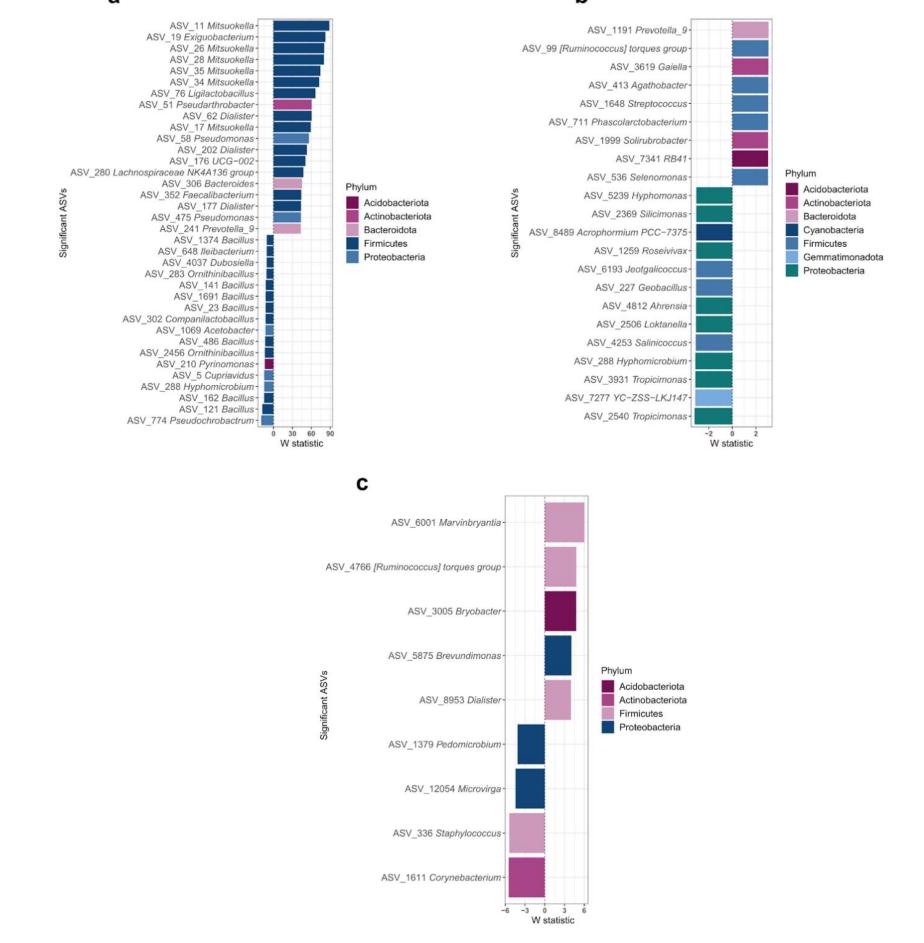


Fig. 4. ANCOM-BC2 differentially abundant bacteria in *D. abudafur* **a)** skin; **b)** gut; **c)** and gills after probiotic treatment. Positive or negative W statistics indicate increased or decreased of bacterial taxa in treated fish.

Significant increase in potentially beneficial bacteria across tissues.

3. Compositional Changes in



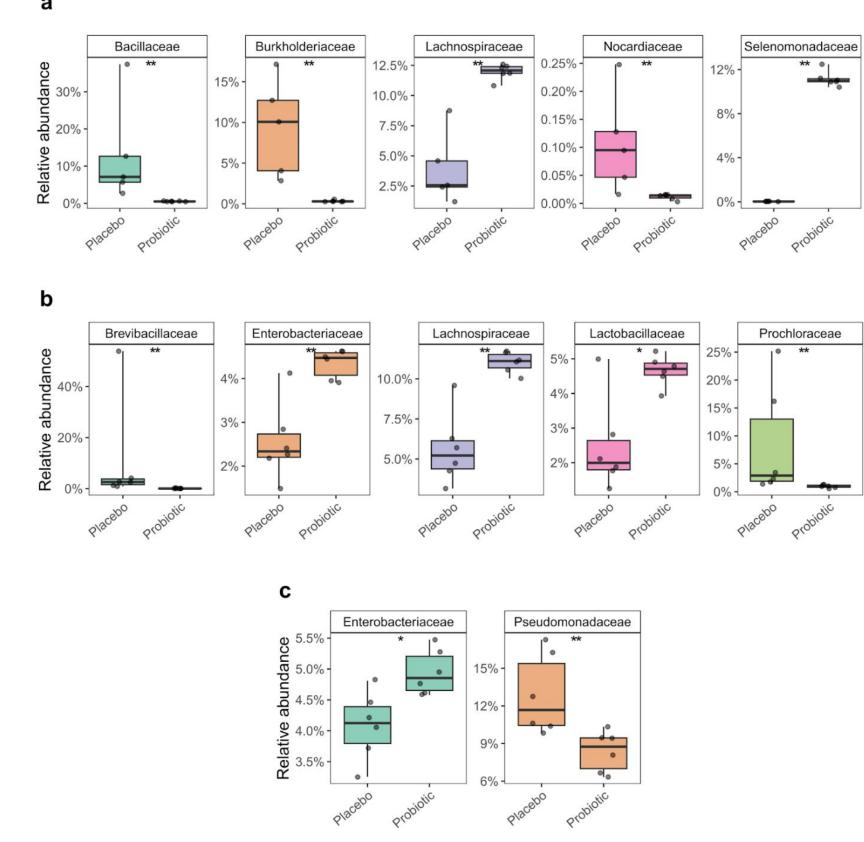


Fig. 3. Changes in top 10 bacterial families in *D. abudafur* tissues post-probiotics (T2). Significant differences between placebo and probiotic-treated corals in the **a)** skin, **b)** gut, and **c)** gills tissue are marked: **p < 0.01; ***p < 0.001.

- Enrichment of beneficial families
 (Lachnospiraceae, Lactobacillaceae) in skin and gut.
- Decrease in potential pathogens (Bacillaceae, Burkholderiaceae).

5. ANCOM-BC2 Skin Pathogen ASVs

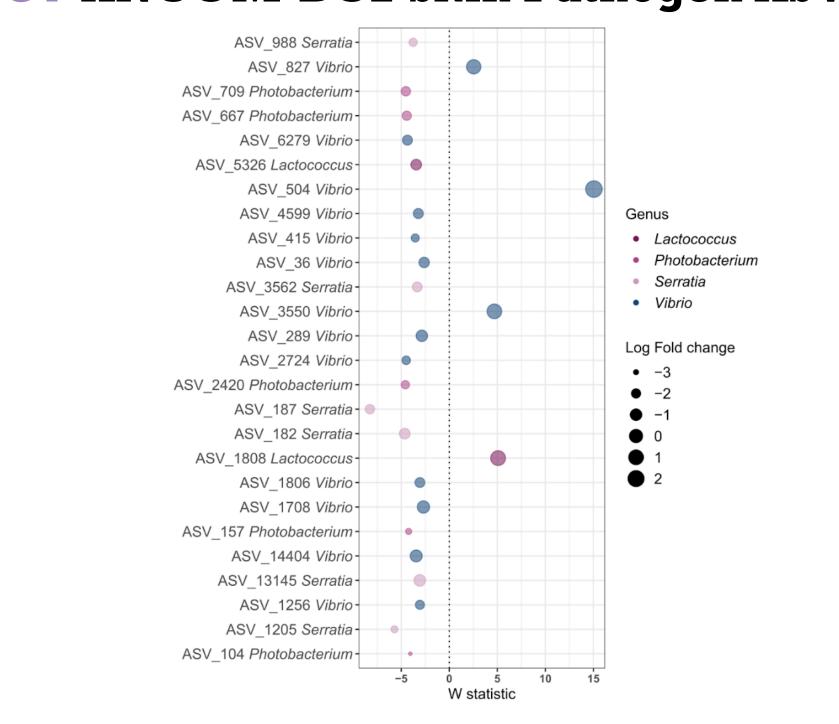


Fig. 5. ANCOM-BC2 differentially abundant ASVs in the skin microbiome linked to potential fish pathogens. Color-coded by genus, with W statistic indicating decreased or enriched taxa in probiotic-treated fish.

 Reduced ASVs of potential pathogens (Vibrio, Photobacterium, Serratia).

CONCLUSION

- <u>Microbiome Restructuring</u>: Probiotic treatment for corals (BMCs) positively reshaped the microbiome of *D. abudafur*, particularly in skin and gut tissues.
- Ecosystem Benefit: The reduction in potential pathogens suggests probiotics' broader protective effect on reef health, aligning with the "One Health" concept.









