NOAA Coral Reef Conservation Program

Award Number NA19NOS4820114

Final Research Performance Report

December 31, 2023

Project Title: Developing Probiotic Treatments to Treat Disease and Protect Captive Coral for Conservation Efforts

Principal Investigator(s):

Dr. Blake Ushijima

Dr. Valerie J. Paul

Smithsonian Marine Station

701 Seaway Drive

Ft. Pierce, FL 34949

Reporting Period September 1, 2019 - August 31, 2023

BACKGROUND

Currently, there is a disease-related coral mortality event occurring on the reefs of Florida that has already resulted in massive die-offs in multiple coral species (Precht *et al.*, 2016; Walton *et al.*, 2018). At least 20 species of corals have displayed sub-acute to acute tissue loss lesions with heavily impacted species being reduced to <3% of their initial population densities (Precht *et al.*, 2016; Florida Keys National Marine Sanctuary, 2018; Walton *et al.*, 2018). The disease, termed stony coral tissue loss disease (SCTLD), was first observed in September 2014 near Miami (Virginia Key) and has since spread to the rest of Florida's Coral Reef (Precht *et al.*, 2016) and across the Caribbean (https://www.agrra.org/coral-disease-outbreak/). The epidemiology of the disease (high rate of spread among low-density hosts) suggests that SCTLD may be caused by an infectious waterborne agent. Laboratory experiments conducted by our research group with diseased corals demonstrated that SCTLD is transmissible through physical contact or contaminated seawater while lesions can normally be treated with antibiotics, suggesting an infectious, possibly bacterial, etiological agent (Aeby *et al.*, 2019). Although the identity of the infectious agent(s) is unclear, antibiotics seem to slow or arrest disease progression (Aeby *et al.*, 2019; Neely *et al.*, 2020; Shilling *et al.*, 2021; Walker *et al.*, 2021), suggesting bacterial pathogens play a role.

Unfortunately, *in situ* treatments using bleach or antibiotic-infused pastes appear to only be temporary treatments for coral colonies in SCTLD endemic zones. Direct treatment of SCTLD lesions with these antibacterial pastes can halt disease progression, but do not provide lasting protection, and corals can be re-infected in another portion of the colony (Walker et al., *pers. comm*.). Therefore, there has been a push for the establishment of land-based nurseries to save coral genotypes for future restoration efforts. However, a major risk during these conservation efforts, as with any captive animal population, is the inadvertent spread of disease because of the high host density held within closed systems. One solution would be to select for genotypes with higher resistance to SCTLD; however, the mechanisms underlying disease resistance and feasible screens are currently unclear.

Interestingly, the disease signs of SCTLD have been observed to vary among affected coral species with differences in the rate of tissue loss (acute and subacute) and lesion occurrence (focal and multi-focal). Other studies have found potential differences in types and levels of defense among coral genera, species and even within species that influence disease occurrence in the field (Mullen *et al.*, 2004; Gochfeld *et al.*, 2006; Roff *et al.*, 2006; Mydlarz *et al.*, 2010). The differences in disease presentation observed between infected corals is hypothesized to be, in part, due to the native microflora present on each colony (Shnit-Orland and Kushmaro, 2009; Rypien *et al.*, 2010; Charlotte *et al.*, 2011; Krediet *et al.*, 2012; Mills *et al.*, 2013). Some of the coral-associated microorganisms that confer protection to their host, probiotics, are believed to produce antimicrobial compounds, which inhibit the growth of or kill putative pathogens (Balcazar *et al.*, 2006; Rypien *et al.*, 2010; Charlotte *et al.*, 2011; Rosado *et al.*, 2018; Ushijima *et al.*, 2023). These probiotic microorganisms could be used to our advantage, especially with the SCTLD outbreak that calls for the development of treatments to reduce mortalities and disease

transmission. Probiotics, for example, could be used to supplement captive corals, thereby reducing the risk of infection and transmission among these vulnerable populations. The care for these captive corals is meticulous, however, the exact etiology of SCTLD is unknown so the actual risks of infection and disease transmission cannot be fully understood.

In contrast to currently used treatments for SCTLD, bleach or antibiotic-infused pastes applied to disease lesions, probiotic treatments could colonize a host and provide lasting protection while being able to be applied to healthy corals (Gatesoupe, 1999; Balcazar *et al.*, 2006). Additionally, growing up batches of probiotics would be more economically feasible than purchasing vast quantities of antibiotics to treat captive corals, especially for more extensive collections. The effectiveness and feasibility of probiotics has been demonstrated in aquatic and terrestrial systems, including humans (Avendaño and Riquelme, 1999; Gatesoupe, 1999; Patterson and Burkholder, 2003; Ibrahim *et al.*, 2004; McFarland, 2009; Kesarcodi-Watson *et al.*, 2012; Zhang and Kim, 2014; Mazanko *et al.*, 2017). Likewise, our preliminary results (see below) suggest that active SCTLD lesions can be slowed or stopped with probiotic treatment and could potentially be used as a prophylactic treatment for captive corals.

RESULTS

Objective #1: To develop probiotic treatments capable of stopping lesion progression on corals affected by SCTLD.

- Task 1A – To optimize the potential probiotics for the treatment of diseased corals.
- Task 1B – To characterize our inhibitory isolate library with a morphological description, inhibitory activity assays, and Sanger sequencing.
- Task 1C – To test the efficacy of using mixtures of probiotic strains in arresting disease progression.

For Task 1A, we have developed an experimental framework to characterize and test coral probiotic treatments for SCTLD, which has been published in the journal *Communications Biology*. The publication describes our first coral probiotic *Pseudoalteromonas* sp. McH1-7:

Ushijima B, Gunasekera SP, Meyer JL, Tittl J, Pitts KA, Thompson S, Sneed JM, Ding Y, Chen M, Jay Houk L, Aeby GS, Häse CC, Paul VJ. 2023. Chemical and genomic characterization of a potential probiotic treatment for stony coral tissue loss disease. *Commun Biol* 6:248.

This publication describes our step-by-step experimental design for:

1) Culturing isolates for testing.
2) Screening microbes for antibacterial activity.
3) Physiological, chemical, and genomic-based characterization of probiotic candidates.
4) Testing probiotic candidates on corals for disease treatment.
5) Testing probiotics candidates on corals for prophylactic potential.
6) Assessing how a probiotic candidate affects the coral microbiome and tracking presence over time.

For Task 1B, we report that we have screened nearly 6,000 microbial isolates from healthy and SCTLD-resistant corals using the methods described in Ushijima et al. 2023 (**Figure 1**). Laboratory screens for isolates that produce antibacterial compounds were conducted on seawater agar (SWA) (described in (Ushijima *et al.*, 2023)) using liquid cultures grown for 24 h. A drop culture assay was used by spotting the culture tested for antibacterial activity (tester strain) onto an SWA plate spread with an isolate that would have their growth inhibited (target strain). The target strains used for the inhibition assays were *Vibrio coralliilyticus* strain OfT6-21, *Alteromonas* sp. strain McT4-15, and *Leisingera* sp. strain McT4-56, which were previously isolated from *O. faveolata* and *M. cavernosa* coral fragments infected with SCTLD during transmission experiments described in a pervious study (Ushijima *et al.*, 2020). After one target strain was spread onto a SWA plate, the tester strains were then spotted, allowed to dry, and then incubated for 24 h. Each screen was done in triplicate with each of the three target strains. After incubation, the zone of inhibition (ZOI) was from the edge of the target strain spot directly to the edge of growth inhibition (if present) (**Figure 2**).

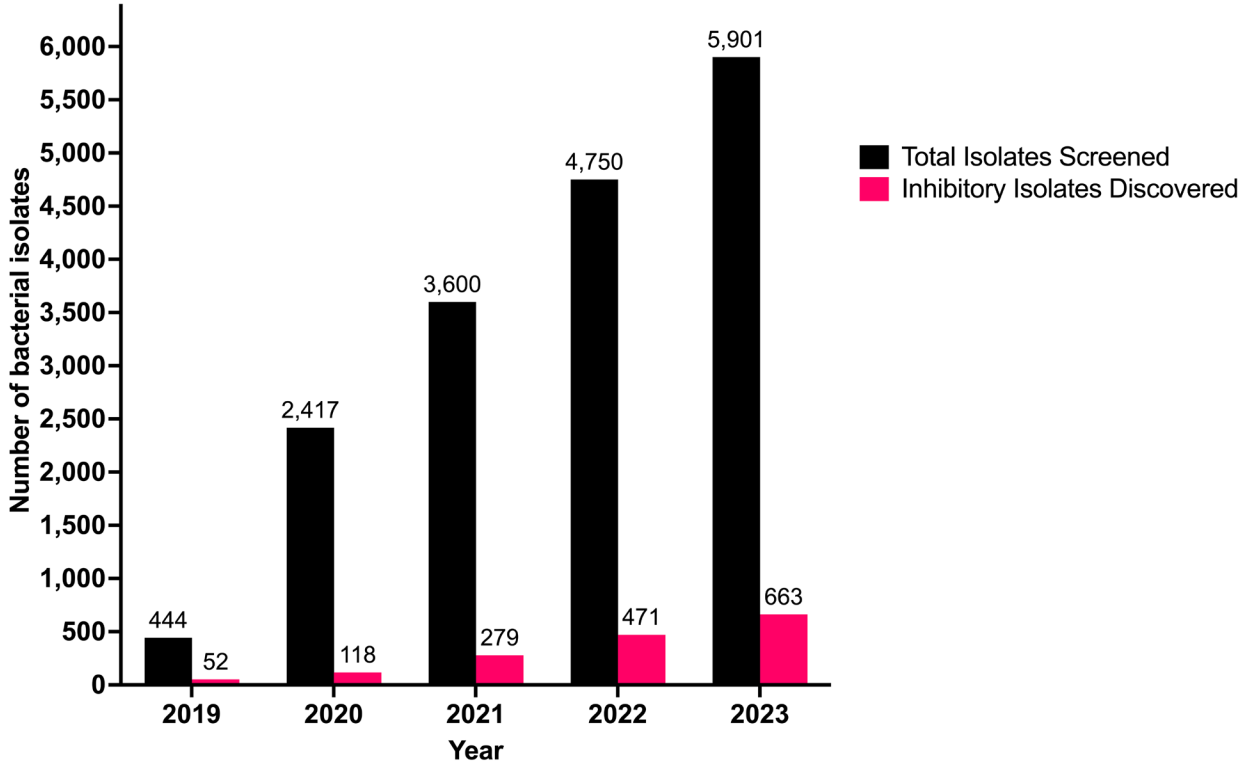

Figure 1. The running total amount of isolates tested. The black bars represent the cumulative total number of isolates that were screened after each year, and the pink bars represent the cumulative total number of isolates discovered with antibacterial activity.

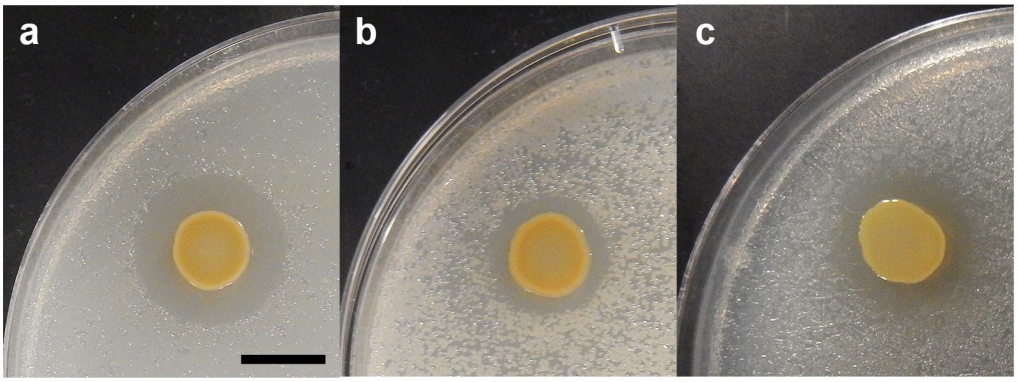

Figure 2. Example of a drop culture assay with McH1-7 and the three target strains. Zone of inhibition (ZOI) of McH1-7 spotted onto target strains A) *Alteromonas* sp. McT4-15, B) *Leisingera* sp. McT4-56, or C) *Vibrio coralliilyticus* OfT6-21. The black scale bar represents 10 mm. *Figure adapted from Ushijima et al. (2023) Chemical and genomic characterization of a potential probiotic treatment for stony coral tissue loss disease. Commun Biol 6: 248.*

We have made significant progress over the duration of this project period and a total of 5,901 isolates have been screened and 663 isolates have been discovered to have antibacterial activity. From those isolates, 18 were selected based on their inhibitory activity, if their antibacterial compounds could be extracted and identified, and did not appear to be related to a pathogenic bacterial species (**Table 1**).

For the coral experiments, diseased corals were collected, fragments and tested as described in our publication (Ushijima *et al.*, 2023). Coral fragments were grouped by colony origin, so that each experimental replicate used fragments from the same colony, i.e., genetically identical specimens. All coral fragments were repeatedly photographed every other day and the area of living tissue remaining was measured using ImageJ (example of progression in **Figure 3**). The total area of living tissue on each fragment was measured at the start of the experiment (day 0), 24 h post, and then every other day after that. Each measurement in cm^2 was divided by the measurement from day 0 and multiplied by 100 to calculate the percent remaining tissue at each time point to standardize the data for the unequal sizes of the coral fragments. The percent of tissue remaining was plotted over time and then the area under the curve (AUC) was calculated for each fragment, with a lower AUC value corresponding to faster disease progression (example of data with McH1-7 in **Figure 3**). The AUC values for the control and experimental fragments were compared using a paired t-test. From these isolates tested on corals, the two isolates that showed the most promise, and therefore our efforts were concentrated on, were McH1-7 and Cnat2-18.1. These two isolates appeared to have the greatest effect on SCTLD lesions and AUC calculations were significantly different than the control corals (paired t-test). Both isolates were identified as species belonging to the bacterial genus *Pseudoalteromonas*, which is know to contain numerous species that produce bioactive compounds that includes antibacterial, antifouling, and anticancer compounds (Bowman, 2007).

Table 1. List of all probiotic candidates tested on diseased corals.

Species isolated from/trialed on	16S rRNA gene seq taxonomy	Strain ID	Sample size	AUC p value (against control)
C. natans	*Halomonas sp*	Cn5-12	6	0.723
C. natans	*Tenacibaculum sp.*	Cn5-34	5	0.226
C. natans	*Pleionea sp.*	CnH1-48	6	0.084
C. natans	*Pseudoalteromonas sp.*	Cnat2-18.1	7	0.008*
M. cavernosa	*Pseudoalteromonas ruthenica*	CnMc7-13	8	0.123
M. cavernosa	*Pseudoalteromonas sp.*	CnMc7-15	17	0.112
M. cavernosa	*Pseudoalteromonas sp.*	CnMc7-37	5	0.516
M. cavernosa	*Pseudoalteromonas sp.*	McH1-7	22	0.002*
M. cavernosa	*Halomonas sp.*	McH1-25	8	0.463
M. cavernosa	*Pseudoalteromonas sp.*	SMS1	6	0.4
M. cavernosa	*Pseudoalteromonas rubra*	XMcav2-N-2	4	0.929
M. cavernosa	*Pseudoalteromonas piscicida*	Xmcav11-Q	2	0.449
O. faveolata	*Pseudoalteromonas sp.*	Of7M-16	12	0.079
Orbicella sp.	*Pseudoalteromonas sp.*	Of5H-5	7	0.542
Orbicella sp.	*Pseudoalteromonas sp.*	Dl2H-1	4	0.939
Orbicella sp.	*Pseudoalteromonas sp.*	Dl2H-2.2	6	0.493
S. siderea	*Vibrio harveyi*	SSH13-20	5	0.229
S. siderea	*Tenacibaculum mesophilum*	SSH1-16	3	0.683

(*) = AUC values are statistically (paired t-test) different than control (untreated) corals.

Separate from SCTLD treatment, we also took the opportunity to run pilot trials with McH1-7 to see if it had any beneficial effects on captive corals experience non-SCTLD related issues. Pilot trials with diseased captive *M. cavernosa* (does not appear to be SCTLD) in Dr. Joana Figueiredo's lab at Nova Southeastern University (NSU) were started February 18 & March 1, 2021, working with veterinarian Dr. Roy Yanong (University of Florida). A total of 13 *M. cavernosa* colonies with progressive tissue loss had been treated with probiotics. Seven of the colonies were previously treated with amoxicillin, ampicillin, or oxolinic acid and had stopped feeding but have not responded to those treatments. McH1-7 was mixed with their standard coral feed (~10^7 cells/ml of food mix) – i.e., 10 mL of a McH1-7 stock (~10^9 cells/ml of seawater) was added to 1 L of food mix. The corals were fed everyday with this mixture. Over the average 47-day treatment period, 11 of the 13 colonies had their lesions arrest and/or began healing. Only one colony died from its disease lesion. Two other colonies died; however, this was due to equipment malfunction and not due to disease. These two colonies appeared to be recovering before the system failure. Another colony had disease stop, but no healing was observed. The remaining nine colonies had disease stop and tissue began healing. This suggests that probiotics like McH1-7 may have utility beyond just a treatment for SCTLD but could have other beneficial properties that could be important for captive corals.

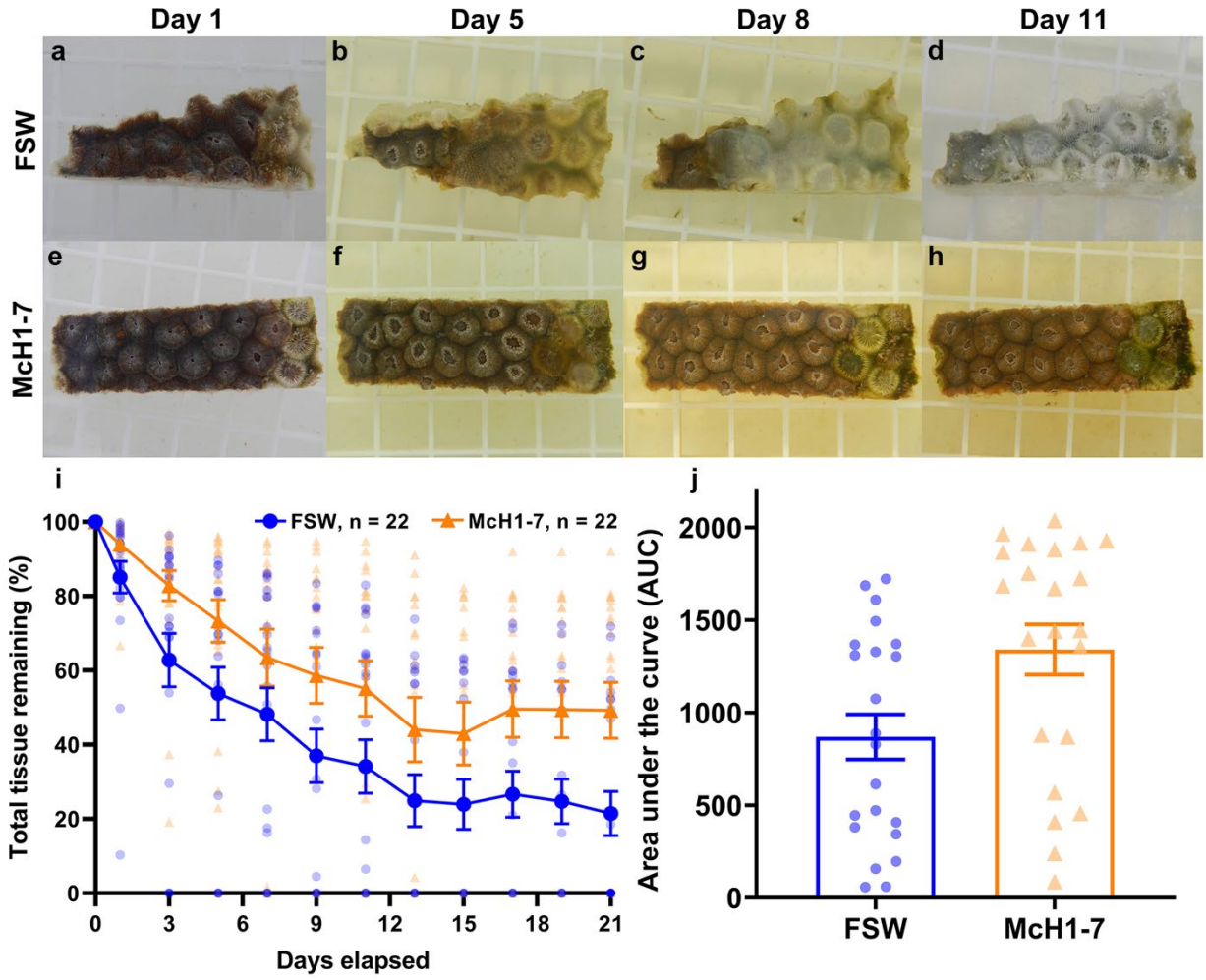

Figure 3. Direct treatment of SCTLD with McH1-7. A-H) Representative photos of diseased *M. cavernosa* treated with filtered seawater (FSW) (A-D) or McH1-7 (E-H). Photos represent days 1, 5, 8, and 11 of the experiment. In this example, the FSW treatment represents 100% tissue loss after 11 days (panel 3D), while the McH1-7 treatment represents 5% tissue loss after 11 days (panel 3H). I) Average percentage of total tissue remaining on *M. cavernosa* colonies treated with FSW (blue circles) or McH1-7 (orange triangles) over time (mixed effects model ANOVA; treatment $p = 0.020$, time $p < 0.0001$, interaction $p = 0.083$; n = 22). The error bars represent the standard error of the mean. J) Area under the curve (AUC) calculations for the diseased *M. cavernosa* treated with FSW or McH1-7 (paired t-test, $p = 0.002$, $n = 22$ each treatment). *Figure from Ushijima et al. (2023) Chemical and genomic characterization of a potential probiotic treatment for stony coral tissue loss disease. Commun Biol 6: 248.*

For Task 1C, to determine if potentially mixing different probiotic strains would improve efficacy, various combinations of strains were trialed with diseased corals by mixing cultures 1:1. This included strains that did not appear as effective on their own, because there was the potential they might work better as a combinational treatment. Bacteria-bacteria antagonism

(using the drop culture assays described above) was measured between potential probiotic mixtures. This was to identify strains that were non- or less- antagonistic to each other so they would have less potential to interfere with one another during treatments. These combination treatments were tested at the same time as the individual strains to compare efficacy, i.e., if the combination treatments did better at stopping/slowing SCTLD progression than the individual strains alone. The lesions in these experiments were analyzed in the same manner described above. The strains SMS1, Of7M-16, CnMc7-15, CnMc7-13, CnMc7-37, DL2H-2.2, DL2H-1 and Cnat2-18.1 were tested in combination treatments with McH1-7 on diseased coral fragments, However, none of the combinations appeared to be as effective or worse than McH1-7 alone. We do not believe that mixtures of probiotics are any more effective than individual strains. This is likely due to the antibacterial activity of each strain or the dilution of individual strains when mixed.

Objective #2: To determine if probiotics are an effective prophylactic treatment that can protect healthy corals from becoming infected.

- Task 2A – To evaluate the use of individual strains and combinations of probiotics to prevent disease transmission to healthy corals.

For objective #2, we have successfully demonstrated that strain McH1-7 can protect healthy corals from SCTLD transmission, which is described in detail within our recent publication (Ushijima *et al.*, 2023). Briefly, diseased and healthy fragments of *M. cavernosa* were split into two and three fragments, respectively. Two of the healthy fragments were inoculated (pre-treated) with McH1-7 and left to sit in an aquarium for 48 h. There were three aquaria per experimental black for this this experiment that were set up: (1) coral control tank that has a pre-treated healthy fragment in contact with another healthy fragment from a different colony to control for intraspecies antagonism, (2) a disease control tank with a non-treated healthy fragment in contact with a diseased fragments, and (3) an experimental tank with a pre-treated healthy fragment in contact with a diseased fragment (**Figure 4**).

There was no obvious damage from intraspecific competition between control corals (**Figure 4a-d**), so any lesions observed with the experimental corals were attributed to disease transmission. The non-treated healthy fragments in contact with diseased fragments (**Figure 4e-h**) had a disease transmission rate of 33.3% (n=12) within 4 to 6 days of contact, which was similar to previous transmission studies (Aeby *et al.*, 2019, 2021). In contrast, none of the fragments pre-treated with McH1-7 (**Figure 4i-l**), developed any disease signs during the 21-day experiment (Log-rank (Mantel-Cox) test, p=0.032, n=12). Surprisingly, the diseased corals in contact with pre-treated fragments had slower disease progression compared to the diseased corals that were in contact with non-treated fragments (mixed effects ANOVA; treatment p=0.004, time p<0.001, interaction p<0.0001; n=12) (**Figure 4m**). The area under the curves (AUC) of tissue loss progression was higher for fragments pre-treated with McH1-7 (Paired t-test, p=0.0001, n=12) (**Figure 4n**). This data suggests that a single treatment with McH1-7 can

protect *M. cavernosa* from SCTLD for at least 21 days and the beneficial effects may be transmissible to diseased corals in aquaria.

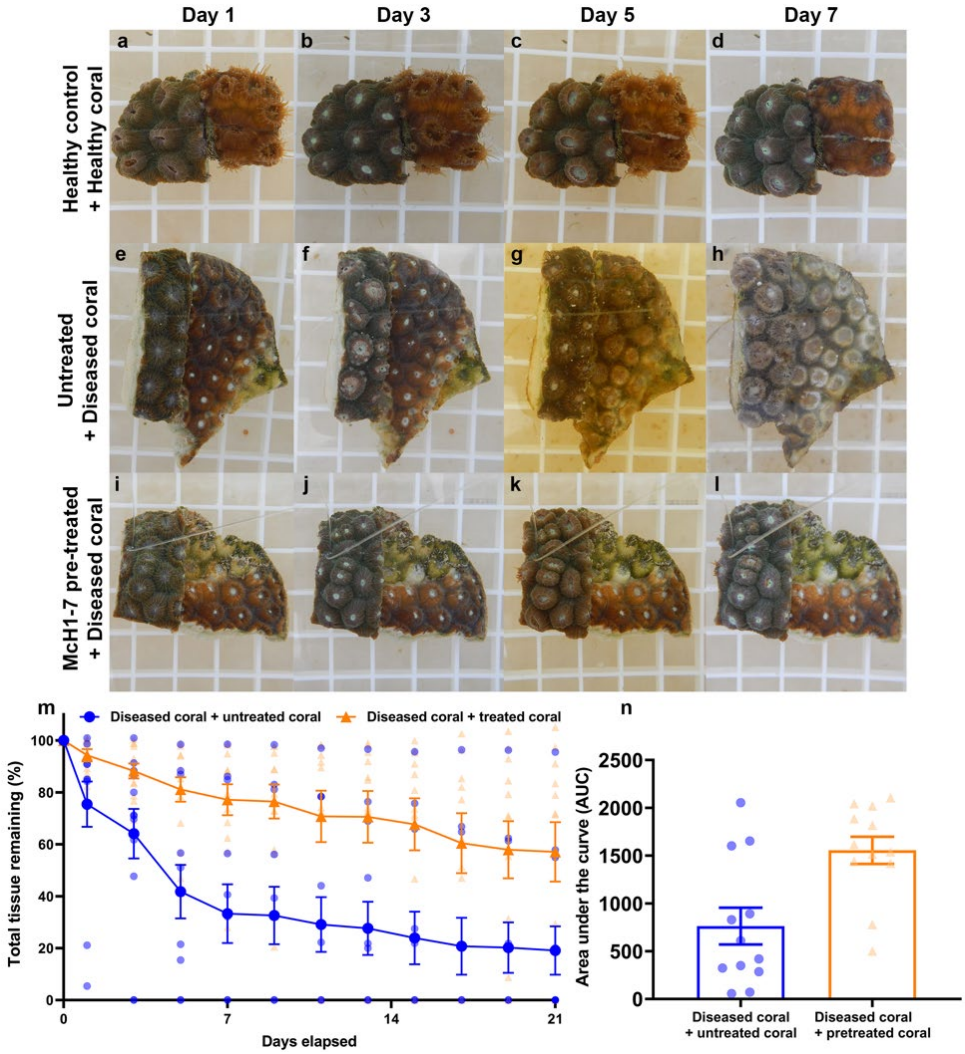

Figure 4. Protection from disease transmission with McH1-7. A-D) photos of control *M. cavernosa* consisting of a healthy fragment pre-treated with McH1-7 (left) in contact with an untreated healthy fragment (right), E-H) untreated healthy fragment (left) in contact with a diseased fragment (right), and I-L) a healthy fragment pre-treated with McH1-7 (left) in contact with a diseased fragment (right). Photos represent days 1, 3, 5, and 7 of the experiment. M) Average percentage of total tissue remaining on diseased *M. cavernosa* colonies in contact with untreated healthy fragments (blue circles) or McH1-7 treated healthy fragments (orange squares) over time (mixed effects model ANOVA; treatment $p = 0.004$, time $p<0.001$, interaction $p<0.0001$; n=12). N) Area under the curve (AUC) calculations for the diseased *M. cavernosa* in contact with untreated fragments (blue bar) or fragments pre-treated with McH1-7 (orange bar) (paired t-test, $p=0.0001$, $n=12$). The error bars represent the standard error of the mean. *Figure from Ushijima et al. (2023) Chemical and genomic characterization of a potential probiotic treatment for stony coral tissue loss disease. Commun Biol 6: 248.*

DELIVERABLES

- We have developed an experimental framework for characterizing and testing coral probiotic candidates, which was published in the journal *Communications Biology* (Ushijima *et al.*, 2023).
- A library of nearly 6,000 total isolates have been isolated, purified, and screened for antibacterial activity (2019 – 2023), with over 600 identified to have antibacterial activity. These strains have been characterized based on general physiology and chemistry as well as identified using Sanger sequencing. Out of the ~600 candidates, 18 isolates had been selected for testing on corals for the efficacy at treating SCTLD directly.
- We have identified two probiotic strains, McH1-7 from *M. cavernosa* and Cnat2-18.1 from *C. natans* that are effective at treating SCTLD in aquarium trials. McH1-7 and Cnat2-18.1 were the most effective and passed safety trials, thus qualifying them as candidates for in situ field trials.
- Probiotic mixtures were determined to not be a viable avenue for treatments because individual strains always performed better than the mixed cultures. This is hypothesized to be due to the antagonistic nature of the probiotics to other microbes or the dilution of each strain when mixed.
- Probiotic strain McH1-7 has been demonstrated to be an effective prophylactic treatment for SCTLD and was able to protect healthy *M. cavernosa* fragments from infection when put into contact with diseased *M. cavernosa* fragments.
- In addition to treating SCTLD, it appears that probiotic McH1-7 could be useful for captive corals as a feed supplement.

IMPLICATIONS FOR MANAGMENT

- The use of beneficial microbes (probiotics) has been identified as a viable treatment for coral disease and stressors like thermal stress (Rosado *et al.*, 2018; Santoro *et al.*, 2021; Ushijima *et al.*, 2023).
- While amoxicillin is currently, effective against SCTLD, it is only a matter of time before the agent develops resistance to this antibiotic like other pathogens. At that point, we will then not have any treatment for SCTLD if more are not developed.
- The creation of an experimental framework for testing probiotics provides an additional avenue for the development of additional treatments for SCTLD and other threats to corals. This allows for a structured approach to develop additional coral disease mitigation treatments.
- The prophylactic effect from probiotics (i.e., the prevention of disease transmission), provides a form of disease protection that is not possible with antibiotics and could serve as an important mitigation strategy for land-based nurseries or in situ efforts.

- The preliminary data for the potential of probiotics being useful as a feed supplement to improve overall coral health does call for more investigation and could be an important aspect for captive corals.

REFERENCES

Aeby, G., Ushijima, B., Bartels, E., Walter, C., Kuehl, J., Jones, S., and Paul, V.J. (2021) Changing Stony Coral Tissue Loss Disease Dynamics Through Time in Montastraea cavernosa. *Front Mar Sci* **8**: 699075.

Aeby, G.S., Ushijima, B., Campbell, J.E., Jones, S., Williams, G.J., Meyer, J.L., et al. (2019) Pathogenesis of a Tissue Loss Disease Affecting Multiple Species of Corals Along the Florida Reef Tract. *Frontiers in Marine Science* **6**:.

Avendaño, R.E. and Riquelme, C.E. (1999) Establishment of mixed-culture probiotics and microalgae as food for bivalve larvae. *Aquaculture Research* **30**: 893–900.

Balcazar, J., Blas, I., Ruizzarzuela, I., Cunningham, D., Vendrell, D., and Muzquiz, J. (2006) The role of probiotics in aquaculture. *Veterinary Microbiology* **114**: 173–186.

Bowman, J.P. (2007) Bioactive compound synthetic capacity and ecological significance of marine bacterial genus Pseudoalteromonas. *Marine Drugs* **5**: 220.

Charlotte, E., Kvennefors, E., Sampayo, E., Kerr, C., Vieira, G., Roff, G., and Barnes, A.C. (2011) Regulation of Bacterial Communities Through Antimicrobial Activity by the Coral Holobiont. *Microbial Ecology* **63**: 605–618.

Florida Keys National Marine Sanctuary (2018) Case Definition: Stony Coral Tissue Loss Disease (SCTLD), NOAA.

Gatesoupe, F.J. (1999) The use of probiotics in aquaculture. *Aquaculture* **180**: 147–165.

Gochfeld, D.J., Olson, J.B., and Slattery, M. (2006) Colony versus population variation in susceptibility and resistance to dark spot syndrome in the Caribbean coral Siderastrea siderea. *Diseases of aquatic organisms* **69**: 53–65.

Ibrahim, F., Ouwehand, A.C., and Salminen, S.J. (2004) Effect of temperature on *in vitro* adhesion of potential fish probiotics. *Microbial Ecology in Health and Disease* **16**: 222–227.

Kesarcodi-Watson, A., Miner, P., Nicolas, J.-L., and Robert, R. (2012) Protective effect of four potential probiotics against pathogen-challenge of the larvae of three bivalves: Pacific oyster (Crassostrea gigas), flat oyster (Ostrea edulis) and scallop (Pecten maximus). *Aquaculture* **344–349**: 29–34.

Krediet, C.J., Ritchie, K.B., Alagely, A., and Teplitski, M. (2012) Members of native coral microbiota inhibit glycosidases and thwart colonization of coral mucus by an opportunistic pathogen. *The ISME Journal* **7**: 980–990.

Mazanko, M.S., Gorlov, I.F., Prazdnova, E.V., Makarenko, M.S., Usatov, A.V., Bren, A.B., et al. (2017) Bacillus Probiotic Supplementations Improve Laying Performance, Egg Quality, Hatching of Laying Hens, and Sperm Quality of Roosters. *Probiotics and antimicrobial proteins* 1–7.

McFarland, L.V. (2009) Evidence-based review of probiotics for antibiotic-associated diarrhea and Clostridium difficile infections. *Anaerobe* **15**: 274–280.

Mills, E., Shechtman, K., Loya, Y., and Rosenberg, E. (2013) Bacteria appear to play important roles in both causing and preventing the bleaching of the coral Oculina patagonica. *Marine Ecology Progress Series* **489**: 155–162.

Mullen, K.M., Peters, E.C., and Harvell, C.D. (2004) Coral resistance to disease. *Structure* **22**: 1.

Mydlarz, L.D., McGinty, E.S., and Harvell, C.D. (2010) What are the physiological and immunological responses of coral to climate warming and disease? *Journal of Experimental Biology* **213**: 934–945.

Neely, K.L., Macaulay, K.A., Hower, E.K., and Dobler, M.A. (2020) Effectiveness of topical antibiotics in treating corals affected by Stony Coral Tissue Loss Disease. *PeerJ* **8**:.

Patterson, J.A. and Burkholder, K.M. (2003) Application of prebiotics and probiotics in poultry production. *Poultry science* **82**: 627–631.

Precht, W.F., Gintert, B.E., Robbart, M.L., Fura, R., and van Woesik, R. (2016) Unprecedented Disease-Related Coral Mortality in Southeastern Florida. *Scientific Reports* **6**: 31374.

Roff, G., Hoegh-Guldberg, O., and Fine, M. (2006) Intra-colonial response to Acroporid "white syndrome" lesions in tabular Acropora spp. (Scleractinia). *Coral Reefs* **25**: 255–264.

Rosado, P.M., Leite, D.C.A., Duarte, G.A.S., Chaloub, R.M., Jospin, G., Nunes da Rocha, U., et al. (2018) Marine probiotics: increasing coral resistance to bleaching through microbiome manipulation. *The ISME Journal*.

Rypien, K.L., Ward, J.R., and Azam, F. (2010) Antagonistic interactions among coral-associated bacteria. *Environmental Microbiology* **12**: 28–39.

Santoro, E.P., Borges, R.M., Espinoza, J.L., Freire, M., Messias, C.S.M.A., Villela, H.D.M., et al. (2021) Coral microbiome manipulation elicits metabolic and genetic restructuring to mitigate heat stress and evade mortality. *Sci Adv* **7**: eabg3088.

Shilling, E.N., Combs, I.R., and Voss, J.D. (2021) Assessing the effectiveness of two intervention methods for stony coral tissue loss disease on Montastraea cavernosa. *Sci Rep* **11**: 8566.

Shnit-Orland, M. and Kushmaro, A. (2009) Coral mucus-associated bacteria: a possible first line of defense. *FEMS Microbiology Ecology* **67**: 371–380.

Ushijima, B., Gunasekera, S.P., Meyer, J.L., Tittl, J., Pitts, K.A., Thompson, S., et al. (2023) Chemical and genomic characterization of a potential probiotic treatment for stony coral tissue loss disease. *Commun Biol* **6**: 248.

Ushijima, B., Meyer, J.L., Thompson, S., Pitts, K., Marusich, M.F., Tittl, J., et al. (2020) Disease Diagnostics and Potential Coinfections by Vibrio coralliilyticus During an Ongoing Coral Disease Outbreak in Florida. *Front Microbiol* **11**: 569354.

Walker, B.K., Turner, N.R., Noren, H.K.G., Buckley, S.F., and Pitts, K.A. (2021) Optimizing Stony Coral Tissue Loss Disease (SCTLD) Intervention Treatments on Montastraea cavernosa in an Endemic Zone. *Front Mar Sci* **8**: 666224.

Walton, C.J., Hayes, N.K., and Gilliam, D.S. (2018) Impacts of a Regional, Multi-Year, Multi-Species Coral Disease Outbreak in Southeast Florida. *Frontiers in Marine Science* **5**:.

Zhang, Z.F. and Kim, I.H. (2014) Effects of multistrain probiotics on growth performance, apparent ileal nutrient digestibility, blood characteristics, cecal microbial shedding, and excreta odor contents in broilers. *Poult Sci* **93**: 364–370.