Testing coral probiotics as an in situ treatment for stony coral tissue loss disease

Prueba de probióticos de coral como tratamiento in situ para la enfermedad de pérdida de tejido de coral pedregoso

Tester les probiotiques coralliens comme traitement in situ du stony coral tissue loss disease

PITTS, KELLY A.[1]; USHIJIMA, BLAKE[2]; MCDONALD, E. MURPHY[3]; DE LA FLOR, YESMARIE[2]; WALKER, BRIAN K.[4]; PAUL, VALERIE J.[1]

[1]Smithsonian Marine Station, 701 Seaway Dr, Fort Pierce, FL 34949, pittsk@si.edu, paul@si.edu; [2]University of North Carolina Wilmington, 601 S College Rd, Wilmington, NC 28403, Ushijimab@uncw.edu, ydelaflor@gmail.com; [3]National Atmospheric and Oceanic Administration, 219 Fort Johnson Rd, Charleston, SC 29412, elizabeth.mcdonald@noaa.gov; [4]Nova Southeastern University, 8000 N Ocean Dr, Dania Breach, FL 32008, walkerb@nova.edu

EXTENDED ABSTRACT

Stony coral tissue loss disease (SCTLD) has spread throughout Florida's Coral Reef causing extensive mortalities in over 21 species of reef-building corals and resulting in the local extinction of some species (1-3). Adding to the mounting devastation by SCTLD in Florida, this disease has recently been found in 17 other countries or territories throughout the Caribbean (https://www.agrra.org/coral-disease-outbreak/). The etiological agents responsible for SCTLD are unknown; however, this disease can be transmitted via contact or through water (4). Current treatments for SCTLD do not provide protection from future infection and may have adverse effects on the local environment. Probiotic treatments represent an opportunity to combat SCTLD using bacteria native to the local environment. Here, we investigate the effectiveness of using coral-derived probiotics as an in situ treatment for SCTLD-affected corals. Bacterial strain McH1-7, isolated from a SCTLD-resistant colony of *Montastraea cavernosa,* inhibited the growth of putative pathogens in the laboratory. Chemical analyses indicated McH1-7 produces korormicin and tetrabromopyrrole, two potential antibacterial compounds. When trialing McH1-7 in aquarium trials, this strain slowed or stopped disease progression on 73% of *Montastraea cavernosa* colonies. To ensure the safety of using McH1-7 on the reef, this strain was tested for safety on a variety of stony coral species and showed no negative effects over 21 days.

To the authors' knowledge, this is the first study to deploy bacterial probiotics on a coral reef. Therefore, it was necessary to develop methodologies of applying probiotics to diseased coral colonies in situ. A plastic bag with weighted line along the bottom was created to treat whole coral colonies. McH1-7, suspended in seawater, was injected via a syringe connected to aquarium tubing up under the bag to treat the coral within. The tubing contained a locking mechanism to ensure McH1-7 was not being released into the surrounding ocean. The bag was left for 2 h to ensure the colonization of McH1-7 on the infected *M. cavernosa* colonies before removing it. A lesion specific treatment was also created using a sodium alginate-based paste with non-toxic, biodegradable thickeners as a vehicle for McH1-7 to be applied over infected tissue. The paste polymerizes, or thickens, when in contact with the divalent ions in seawater to allow the paste to better stick to the coral lesion. Using these methodologies, we tested five different treatments on *Montastraea cavernosa* colonies on a reef off the coast of Broward County, FL: 1) probiotic paste that involved coating the SCTLD lesion in paste containing a high concentration of McH1-7; 2) control paste which involved the same protocol as the probiotic paste but absent of probiotics; 3) probiotic bag for covering the coral with a plastic bag, injecting McH1-7 and leaving the bag for 2 h; 4) control bag, which is the same as the probiotic bag treatment, but injecting seawater instead of McH1-7; and 5) background control where diseased corals were not treated, but monitored over time.

Corals were treated on September 1, 2020; October 14, 2020; and January 15, 2021. The corals were photographed each time and once again photographed on February 25, 2021 for signs of disease progression. Each colony was categorized into 1 of 5 health statuses based on photographs taken on February 25th, 2021, after most corals had been treated 3 times (Fig. 1): Healing = bleached tissue is regaining zooxanthellae and pigmentation; stopped = the disease has stopped progressing across the coral and tissue loss has stopped; chronic tissue loss = the disease is slowly progressing across the coral; acute tissue loss = the disease is quickly progressing across the coral; dead = all of the tissue on the colony has died. There is a significant association between health status and treatment for these categories (Fisher exact test: $p = 0.002$). A total of 75% of the corals treated with the probiotic bag had healed, whereas disease had stopped progressing on the remaining 25%.

Three-dimensional (3D) models were created of each coral for each date the site was visited to compare disease progression over time. Using Professional Agisoft Metashape, the healthy tissue remaining (cm^2) was measured on the 3D model of each coral. Although results were not significant, it appears that the corals treated with a probiotic bag or probiotic paste were losing the least amount of healthy tissue over time compared to control treatments (Fig. 2). A larger sample size is needed to better understand differences between treatments so two more sites have been established off the coast of southern Florida. All three sites will continue to be monitored over time. In all, these results represent the first bacterial probiotic trials in a coral reef system and suggest that this is a feasible treatment for SCTLD and further studies should be conducted.

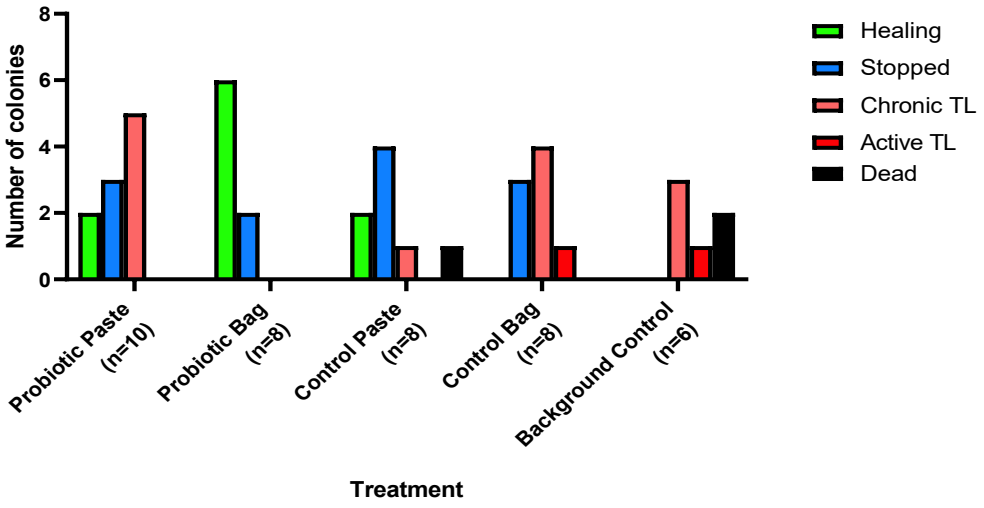

Figure 1. . Number of coral colonies healing (green), with stopped lesion progression (blue), slow lesion progression (pink), fast lesion progression (red), or completely dead (black) based on the treatment they received

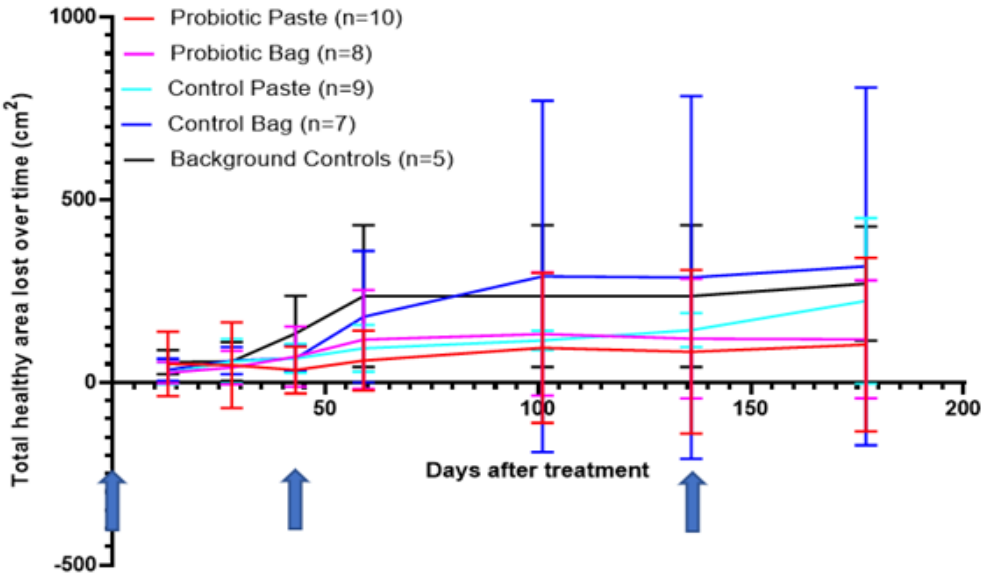

Figure 2. Total healthy tissue surface area (cm^2) lost on corals at BS2 over time. Corals were treated on day 0, 43, and 136 (blue arrows). Data are shown as mean ± 1 SEM.

KEYWORDS: Probiotics, SCTLD, coral disease, *Montastraea cavernosa* .

LITERATURE CITED

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

Florida Keys National Marine Sanctuary, "Case Definition: Stony Coral Tissue Loss Disease (SCTLD)" (Case Definition October 2018, NOAA, 2018), pp. 1–10.

Muller, E.M. C. Sartor, N. I. Alcaraz, R. van Woesik, Spatial Epidemiology of the Stony-Coral-Tissue-Loss Disease in Florida. *Front. Mar. Sci.* **7**, 163 (2020).

Aeby, G.S. B. Ushijima, J. E. Campbell, S. Jones, G. J. Williams, J. L. Meyer, C. Häse, V. J. Paul, Pathogenesis of a tissue loss disease affecting multiple species of corals along the Florida reef tract. *Front. Mar. Sci.* 6:678 (2019).