Rhodobacterales, Rhizobiales, Desulfovibrionales, and Clostridiales are significant taxa associated with stony coral tissue loss disease in *Montastraea cavernosa* and *Orbicella faveolata*

Rhodobacterales, Rhizobiales, Desulfovibrionales y Clostridiales son taxones importantes asociados con la enfermedad de pérdida de tejido de coral pétreo en *Montastraea cavernosa* y *Orbicella faveolata*

Les Rhodobacterales, les Rhizobiales, les Desulfovibrionales et les Clostridiales sont des taxons importants associés à la maladie de la perte de tissu des coraux durs chez *Montastraea cavernosa* et *Orbicella faveolata*

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EXTENDED ABSTRACT

Over the last several decades, coral disease outbreaks have caused substantial declines in live coral cover in the Caribbean region. Particularly, coral disease outbreaks are a primary contributor to the coral decline that Florida's Coral Reef (FCR) has experienced. Coral diseases was first documented within FCR in the 1970s, and several disease outbreaks have been recorded with increased frequency since this time (Porter et al., 2001; Santavy et al., 2001; Borger 2005).

Since 2014, FCR has experienced a multi-year disease related mortality event termed stony coral tissue loss disease (SCTLD). This disease was first recorded off Virginia Key, Florida (Miller et al., 2016; Precht et al., 2016; Walton et al., 2018) and has since spread throughout the entire FCR and other areas of the Caribbean (Alvarez-Filip et al., 2019; Heres et al., 2021; Weil et al., 2019). This disease impacts over half of the reef-building species in Florida, has high rates of mortality and follows a contagious model of transmission (Muller et al., 2020). Despite this, researchers have been unable to determine definitive pathogen(s) for this disease. Some studies have indicated that SCTLD lesion progression can be slowed down or halted on some coral species with amoxicillin paste (Aeby et al., 2019; Neely et al., 2020; Shilling et al., 2021), suggesting that the presumptive pathogen(s) for SCTLD may have a bacterial component. Other studies have used 16S ribosomal RNA (rRNA) sequencing approaches to identify differences in bacterial communities between apparently healthy and SCTLD affected corals (Meyer et al., 2019; Rosales et al., 2020; Becker et al., 2021; Clark et al., 2021; Iwanowicz et al., 2021). However, whether the identified bacteria are primary pathogens or secondary opportunistic infections is still unknown. Here, we used 16S rRNA sequencing to identify key differences in the bacterial community of apparently healthy and SCTLD-affected *Montastraea cavernosa* and *Orbicella faveolata* fragments in an experimental setting. Additionally, we examined how the bacterial community changed over time within a diseased fragment, and compared this change over time between the two coral species. Lastly, we compared the identified disease associated bacterial taxa with disease associated taxa identified from previous SCTLD microbiome studies.

Samples were collected from two disease transmission experiments conducted in the Coral Health and Disease wetlab at Mote Marine Laboratory (MML) in Sarasota, FL as described in Eaton et al. (2021). Briefly, the experiments were conducted as follows: The first transmission experiment was conducted from April–June 2019 using apparently healthy and diseased *M. cavernosa*. The second transmission experiment was conducted from July– September 2019 using apparently healthy *O. faveolata* and diseased *M. cavernosa*. For both experiments, "apparently healthy corals" were defined as showing no visually obvious signs of tissue loss or stress. "Diseased corals" were defined as showing a grossly-visible (subacute) tissue loss lesion. For each experiment, apparently healthy coral colonies were collected from the Airport Coral Heads site in Key West (24.53919°, −81.77270°) which was ahead of the known SCTLD zone at the time. Diseased colonies were collected at Looe Key (24.54767°, −81.45697°). Colonies were then transported to the Coral Health and Disease wetlab at MML in Sarasota, FL for experimentation. Upon arrival, apparently healthy and diseased corals were fragmented, and each apparently healthy experimental coral fragment (referred to as "recipient" fragment) was put into physical contact with a disease fragment or apparently healthy control fragment (referred to as "donor" fragment).

Samples for microbiome analyses were collected by scraping the surface of the lesioned area with a sterile razor blade when recipient fragments experienced approximately 5%, 25%, 50%, and 75% tissue loss. As described in Eaton et al. (2021) parallel samples were taken for histological analyses to verify hallmark histopathologic lesions of SCTLD per Landsberg et al. (2020). Each disease sample collected was paired with an apparently healthy sample collected from a recipient fragment within a control tank. Samples were then flash frozen and stored in a -80 °C freezer. DNA was extracted

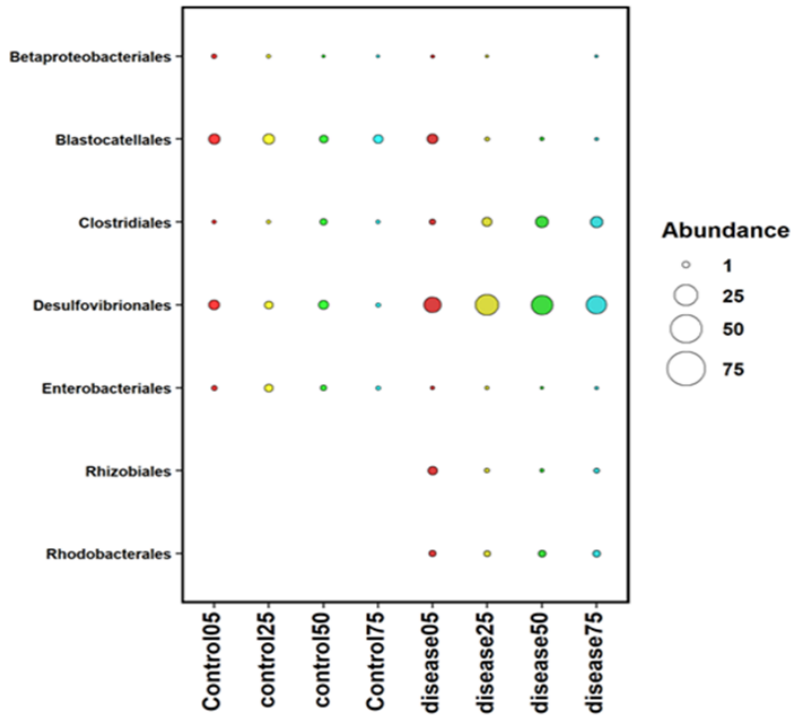

Figure 1. Relative abundances of significantly differentiated taxa among the *M. cavernosa* at different disease progression points, and associated control samples. Taxa were grouped by order.

using a DNeasy PowerSoil Kit (QIAGEN, Germantown, MD, USA) with modifications to the manufacturer's protocol (see Rosales et al. 2020). DNA isolates were sequenced on the Illumina MiSeq platform for 16S rRNA to determine bacterial community dynamics (MR DNA, www.mrdnalab.com, Shallowater, TX, USA). Amplification of the 16S rRNA gene was conducted using PCR primers 515F (GTGCCAGCMGCCGCGGTAA; Original Earth Microbiome Project; Caporaso et al., 2011) and 806R (GGACTACVSGGGTATCTAAT; Archaea 806R; Takai and Horikoshi, 2000) with barcode on the forward primer. The DADA2 pipeline (Callahan et al., 2016) was used to determine amplicon sequence variants (ASVs) by checking read quality, filtering and trimming sequences, dereplicating, merging sequences, and removing chimeras. Taxonomy was assigned using the SILVA reference database (version 132; European Organization for Nuclear Research, Geneva, Switzerland). Analyses of composition of microbiomes (ANCOMs, Mandal et al., 2015) were performed to determine which bacterial families were differentially abundant between diseased and apparently healthy control corals at each progression point for each experiment.

Overall, several bacterial taxa were repeatedly more abundant within experimentally-induced diseased tissues compared with healthy tissue samples. In both species, orders Rhodobacterales, Rhizobiales, Desulfovibrionales and Clostridiales were found at higher relative abundances in disease samples compared to apparently healthy control samples (**Figures 1 and 2**). In *M. cavernosa,* the relative abundances of Clostridiales and Desulfovibrionales increased as the disease progressed. Also, Rhodobacterales and Rhizobiales were not present in apparently healthy control *M. cavernosa* samples (**Figure 1**). In *O. faveolata*, the relative abundance of Clostridiales increased as the disease progressed. Also, Chlamydiales were consistently more abundant in apparently healthy control *O. faveolata* samples (**Figure 2**), perhaps a part of this coral species core microbiome. Relative abundances of Rhodobacterales, Rhizobiales, and Desulfovibrionales were consistent through time in both species (**Figures 1 and 2**). Although, there were significant differences in the bacterial communities of diseased *M. cavernosa* and *O. faveolata* through time, these significant differences were primarily driven by the presence of Cytophagales, Coxiellales, Desulfobacterales, and Legionellales in diseased *O. faveolata* tissue.

The primary disease associated taxa found in this study have been documented in other SCTLD microbiome studies. Rhodobacterales was documented in all previous SCTLD microbiome studies (Meyer et al., 2019; Rosales et al., 2020; Becker et al., 2021; Clark et al., 2021; Iwanowicz et al., 2021), Rhizobiales was documented in Rosales et al. (2020) and Becker et al. (2021), Clostridiales identified in Meyer et al. (2019), Clark et al. (2021), and Iwanowicz et al. (2021), and Desulfovibrionales was identified in Becker et al. (2021). The consistency in identified taxa within SCTLD samples across multiple studies, and replicated here experimentally, suggests that the taxa Rhodobacterales, Rhizobiales, Desulfovibrionales, and Clostridiales play a critical role in disease dynamics of

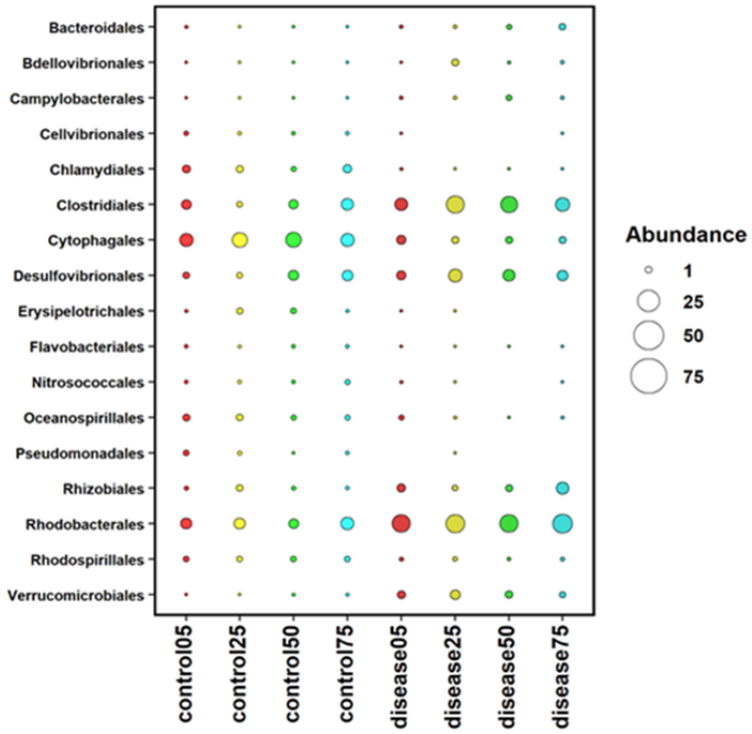

Figure 2. Relative abundances of significantly differentiated taxa among the *O. faveolata* at different disease progression points, and associated control samples. Taxa were grouped by order.

SCTLD. Additionally, the taxa identified in this study are associated with other coral diseases. Rhodobacterales, Rhizobiales, Clostridiales, and Desulfovibrionales have been associated with black band disease (Cooney et al., 2002; Sekar et al., 2006; Viehman et al., 2006). Rhodobacterales, Rhizobiales, and Clostridiales have been associated with white plague, white band, and/or white syndrome (Pantos et al., 2003; Pantos et al., 2006; Sunagawa et al., 2009). Thus, the results presented here suggest that next steps should focus on: 1) identifying consistencies at the ASV level across all SCTLD microbial studies, including the experimental data collected here (currently ongoing) 2) studying the function of these taxa in disease progression using metagenomics 3) the potential to isolate these bacteria in culture to attempt inoculation studies, and 4) the development of targeted therapies that remove individual taxa from diseased microbiomes to test for treatment.

KEYWORDS: Florida's coral reef, stony coral tissue loss disease, microbiome, *Montastraea cavernosa, Orbicella faveolata*

LITERATURE CITED

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. *Front. Mar. Sci.* 6: 1–18. doi: 10.3389/fmars.2019.00678

Alvarez-Filip, L., Estrada-Saldívar, N., Pérez-Cervantes, E., Molina-Hernández, A. and González-Barrios, F. J. (2019). A rapid spread of the stony coral tissue loss disease outbreak in the Mexican Caribbean. *PeerJ* 7: e8069. doi: 10.7717/peerj.8069

Becker, C. C., Brandt, M., Miller, C. A. and Apprill, A. (2021). Microbial bioindicators of Stony Coral Tissue Loss Disease identified in corals and overlying waters using a rapid field-based sequencing approach. *Environ. Microbiol*. doi: 10.1111/1462-2920.15718

Borger, J. L. (2005). Scleractinian coral disease in south Florida: incidence, species susceptibility, and mortality. *Dis. Aquat. Org.* 67: 249–258. doi:10.3354/dao067249

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016). DADA2: high-resolution sample inference from Illumina amplicon data. *Nat. Methods* 13: 581–583. doi: 10.1038/nmeth.3869

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., et al. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *PNAS* 4516-4522; doi: 10.1073/pnas.1000080107

Clark, A. S., Williams, S. D., Maxwell, K., Rosales, S. M., Huebner, L. K., Landsberg, J. H. et al. (2021). Characterization of the microbiome of corals with stony coral tissue loss disease along Florida's Coral Reef. *Microorg.* 9: 2181. doi:10.3390/microorganisms9112181

Cooney, R. P., Pantos, O., Le Tissier, M.D.A., Barer, M. R., O'Donnell, A.G. and Bythell, J.C. (2002). Characterization of the bacterial consortium associated with Black Band Disease in coral using molecular microbiological techniques. *Environ. Microbiol.*, 4: 401–413

Eaton, K. R., Landsberg, J. H., Kiryu, Y., Peters, E. C. and Muller, E. M. (2021). Measuring stony coral tissue loss disease induction and lesion progression within two intermediately susceptible species, *Montastraea cavernosa* and *Orbicella faveolata*. *Front. Mar. Sci*. 8: 717265. doi: 10.3389/fmars.2021.717265

Heres, M. M., Farmer, B. H., Elmer, F. and Hertler, H. (2021). Ecological consequences of Stony Coral Tissue Loss Disease in the Turks and Caicos Islands. *Coral Reefs* 40: 609–624. doi: 10.1007/s00338-021-02071-4

Iwanowicz, D. D., Schill, W. B., Woodley, C. M., Bruckner, A., Neely, K. and Briggs, K. M. (2020). Exploring the Stony Coral Tissue Loss Disease bacterial pathobiome. *bioRxiv*. doi: 10.1101/2020.05.27.120469

Landsberg, J. H., Kiryu, Y., Peters, E. C., Wilson, P. W., Perry, N., Waters, Y. et al. (2020). Stony coral tissue loss disease in Florida is associated with disruption of host–zooxanthellae physiology. *Front. Mar. Sci.* 7: 576013 doi: 10.3389/fmars.2020.576013

Mandal S., Van Treuren W., White R. A., Eggesb M., Knight R., Peddada S. D. (2015). Analysis of composition of microbiomes: a novel method for studying microbial composition. *Microb. Ecol. Health Dis*. 26: 1, 27663. doi: 10.3402/mehd.v26.27663

Meyer, J. L., Castellanos-Gell, J., Aeby, G. S., Häse, C. C., Ushijima, B., and Paul, V. J. (2019). Microbial community shifts associated with the ongoing stony coral tissue loss disease outbreak on the Florida reef tract. *Front. Microbiol.* 10: 2244. doi: 10.3389/fmicb.2019.02244

Miller, M. W., Karazsia, J., Groves, C. E., Griffin, S., Moore, T., Wilber, P., et al. (2016). Detecting sedimentation impacts to coral reefs resulting from dredging the Port of Miami, Florida USA. *PeerJ* 4: e2711. doi: 10.7717/peerj.2711Muller, E. M., Sartor, C., Alcaraz, N. I. and van Woesik, R. (2020). Spatial epidemiology of the stony-coral-tissue-loss disease in Florida. *Front. Mar. Sci.* 7: 163. doi: 10.3389/fmars.2020.00163

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: e9289. doi: 10.7717/peerj.9289

Pantos, O., Cooney, R. P., Le Tissier, M. D.A., Barer, M.R., O'Donnell, A. G. and Bythell, J. C. (2003). The bacterial ecology of a plague-like disease affecting the Caribbean coral *Montastrea annularis*. *Environ. Microbiol.* 5: 370–382.

Pantos, O. and Bythell, J. C. (2006). Bacterial community structure associated with white band disease in the Elkhorn coral *Acropora palmata* determined using culture-independent 16S rRNA techniques. *Dis. Aquat. Org.*, 69: 79–88.

Porter, J. W., Dustan, P., Jaap, W. C., Patterson, K. L., Kosmynin, V., Meier, O. W. et al. (2001). Patterns of spread of coral disease in the Florida Keys. In: *The Ecology and Etiology of Newly Emerging Marine Diseases.* Springer, pp 1–24

Precht, W. F., Gintert, B. E., Robbart, M. L., Fura, R., and Van Woesik, R. (2016). Unprecedented disease-related coral mortality in southeastern Florida. *Sci. Rep.* 6: 31374 doi: 10.1038/srep31374

Rosales, S. M., Clark, A. S., Huebner, L. K., Ruzicka, R. R., and Muller, E. M. (2020). Rhodobacterales and Rhizobiales are associated with stony coral tissue loss disease and its suspected sources of transmission. *Front. Microbiol.* 11: 681. doi: 10.3389/fmicb.2020.00681

Santavy, D. L., Mueller, E., Peters, E. C., MacLaughlin, L., Porter, J. W., Patterson, K. L. et al. (2001). Quantitative assessment of coral diseases in the Florida Keys: strategy and methodology. *Hydrobiol.* 460: 39–52. doi: 10.1007/978-94-017-3284-0_3

Sekar, R., Mills, D. K., Remily, E. R., Voss, J. D. and Richardson, L. L. (2006). Microbial communities in the surface mucopolysaccharide layer and the black band microbial mat of black band-diseased *Siderastrea siderea*. *Appl. Environ. Microbiol. 72*: 5963–5973

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. doi: 10.1038/s41598-021-86926-4

Sunagawa, S., DeSantis, T.Z., Piceno, Y.M., Brodie, E.L., DeSalvo, M.K., Voolstra, C.R. et al. (2009). Bacterial diversity and white plague disease-associated community changes in the Caribbean coral *Montastraea faveolata*. *ISME J.* 3: 512–521

Takai, K., and Horikoshi, K. (2000). Rapid detection and quantification of members of the archaeal community by quantitative PCR using fluorogenic probes. *Appl Environ Microbiol* 66: 5066–5072

Viehman, S., Mills, D. K., Meichel, G. W. and Richardson, L. L. (2006). Culture and identification of *Desulfovibrio* spp. from corals infected by black band disease on Dominican and Florida Keys reefs. *Dis. Aquat. Org.* 69: 119–127.

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. *Front. Mar. Sci.* 5: 323. doi: 10.3389/fmars.2018.00323

Weil, E., Hernández-Delgado, E. A., Gonzalez, M., Williams, S., Suleimán-Ramos, S., Figuerola, M. et al. (2019). Spread of the new coral disease "SCTLD" into the Caribbean: implications for Puerto Rico. *Reef Encounter* 34: 38–43.