Biofilms as a potential transport mechanism for Stony Coral Tissue Loss Disease (SCTLD) into new regions

Las biopelículas como un mecanismo potencial de transporte para la enfermedad de la pérdida de tejido de coral pétreo (SCTLD) en nuevas regiones

Les biofilms comme mécanisme potentiel de transport de la maladie de la perte de tissu corallien sur les coraux durs (SCTLD) dans de nouvelles régions

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EXTENDED ABSTRACT

In 2014, a new coral disease now known as stony coral tissue loss disease (SCTLD) was documented on reefs off the coast of Virginia Key, near the Port of Miami (Precht et al. 2016). SCTLD is unique in the rapidity of its progression, the number of coral species it affects, and the temporal extent of its impact. Yet despite considerable research efforts, the causative agent of SCTLD remains unknown, and substantial debate remains as to whether the disease has a bacterial, viral, or other cause. However, waterborne transmission of SCTLD has been established (Aeby et al. 2019), indicating infected corals must shed the unknown causative agent into the environment. Additionally, antibiotics are effective in treating disease lesions, suggesting bacteria may play a role in disease progression (Aeby et al. 2019; Neely et al. 2020). Additionally, molecular investigations have repeatedly identified specific bacterial taxa in association with SCTLD, with studies spanning different coral species, locations, and collection years (Becker et al. 2021; Clark et al. 2021; Meyer et al. 2019; Rosales et al. 2020).

Over the past 8 years, SCTLD has spread at a relatively linear rate to infect corals throughout the entirety of the Florida Reef Tract, with outbreaks occurring in locations consistent with natural spread via water currents (Muller et al. 2020). In the wider Caribbean, however, outbreaks of the disease have been sporadic, often clustered near ports and in isolated locations (Dahlgren et al. 2021), suggesting ships may also contribute to the spread of the disease. Biofouled ship hulls have long been recognized as a transport vector for nonnative species into new regions, but whether biofilms attached to ship hulls or within ship ballast tanks could serve to introduce microbial pathogens into new areas is less clear. Further, because SCTLD is a waterborne disease, infected corals must shed the unknown causative agent into the surrounding water, but whether these coral-shed microbes are even capable of biofilm formation is also poorly understood. To investigate the potential role of biofilms in aiding the spread of SCTLD, we addressed three research questions: 1) whether coral-shed microbes are capable of forming biofilms; 2) whether we can detect differences between the source corals within these biofilm microbial communities; and 3) whether a SCTLD signal can be identified within these biofilm microbial communities.

To address these questions, 5 gal. bucket mesocosms (n = 15) were filled with approximately 18 L of seawater that had been 0.22 µm filtered and UV-treated to reduce the starting background microbial load. A coral colony was added to mesocosms (n = 14) to serve as a source of microbes. Diseased mesocosms (n = 10) received colonies (*Colpophyllia natans*, n = 9; or *Pseudodiploria strigosa*, n = 1) that had been recently collected from reefs near Vaca Key, Florida, and were exhibiting visual symptoms consistent with SCTLD. Given the active or endemic status of SCTLD on Florida reefs, healthy mesocosms (n = 4) each received colonies (*C. natans*, n = 2; or *P. strigosa*, n = 2) that had been collected from Florida reefs and subsequently held in quarantine for 1-2 years to ensure they were SCTLD-naïve. These apparently healthy colonies exhibited no visual symptoms consistent with SCTLD. The remaining mesocosm (n = 1) received no coral and served as a seawater control. Marine-grade stainless steel plates (~7.5 cm x 5.1 cm) were cleaned, autoclaved, and added to mesocosms (n = 15) to serve as proxies for ship hulls, ballast tank interior walls, etc. Plates were placed in sterile petri dishes to allow for water contact on all sides, exposed to mesocosm water for three days, then removed and preserved in RNAlater. Adhered biofilms were removed by scraping both sides of the plates with sterile razor blades and rinsing with a sterile buffer. DNA was extracted using a Qiagen DNEasy PowerBiofilm kit. To target the bacterial / archaeal biofilm community, the v4 region of the 16S rRNA gene was sequenced on an Illumina MiSeq platform using the universal bacterial / archaeal primers 515F and 806R. Resulting raw sequences were then processed through the QIIME2 platform (v. 2021.4; Bolyen et al. 2019) and quality-filtered and assigned to 100% amplicon sequence variants (ASVs) using DADA2 (Callahan et al. 2016). Taxonomy was assigned to these ASVs using the pre-trained naïve Bayes classifier SILVA-138-99-515-806 (Bokulich et al. 2018). Distance matrices were constructed within QIIME2, and Emperor principal coordinate analysis

(PCoA) plots were generated to visualize the relationships between different samples. Beta-diversity was statistically analyzed using permutational multivariate analyses of variance (PERMANOVA) based on the weighted UniFrac distance matrix.

We determined that coral-shed microbes were capable of biofilm formation and that they produced biofilms consisting of highly diverse microbial communities that looked distinctly different from those of the seawater control. We further determined that differences in the source corals are reflected within the resulting biofilm microbial communities. For example, we detected a significant difference in microbial community structure between healthy and diseased coral biofilms (PERMANOVA; $p < 0.05$). However, given that the influence of individual source coral characteristics may be difficult to distinguish (e.g., healthy corals were sourced from aquaria while diseased corals were sourced from the reef, both factors which may be expected to influence microbial community structure), the finding of health status of the source corals directly contributing to biofilm microbial community structure and composition should be interpreted cautiously. We further detected numerous ASVs previously identified as SCTLD-associated (Becker et al. 2021; Meyer et al. 2019; Rosales et al. 2020) often within exclusively the diseased mesocosms, suggesting that disease state in particular may play a greater role in shaping these biofilm microbial communities than other source coral characteristics. While it is currently unclear if the still-unknown SCTLD pathogen is a member of these biofilm communities, this work suggests biofilms warrant additional investigation as a potentially contributing factor in the spread of SCTLD.

*Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. *

KEYWORDS: SCTLD, Coral, Disease, Biofilm, Bacteria

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