Characterizing the Cell-Associated Bacterial Community of two toxigenic *Pseudo-nitzschia* Species in Culture

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In 2016 and 2017, Narragansett Bay (NB), Rhode Island shellfishing harvests were shut down for the first time due to high levels of neurotoxin domoic acid (DA). Ingestion of DA by humans can cause short-term memory loss, brain damage, and, in severe cases, death. *Pseudo-nitzschia* is a genus of diatom capable of producing DA and is considered a harmful algal bloom (HAB) taxon. In NB, *Pseudo-nitzschia* has been recorded in Narragansett Bay since the 1960s but did not cause toxin-related closures until 2016-17. Species within the *Pseudo-nitzschia* genus vary in toxicity, making HAB forecasting difficult. Additionally, environmental factors, including the bacteria associated with *Pseudo-nitzschia* cells, affect DA production. Research has shown that increases in DA levels are correlated with a decrease in the biodiversity of the cell-associated bacteria community of *Pseudo-nitzschia* in field and culture samples. The correlation between DA levels and the biodiversity of bacteria leads researchers to believe that *Pseudo-nitzschia* produces DA as a deterrent to pathogenic bacteria. NB has a mix of toxic and non-toxic species, with two of the most common being the toxin producers, *P. pungens* and *P. multiseries*. In culture, isolates of the two species produce variable amounts of DA or do not produce the toxin. In this project, we assessed the growth rate of *Pseudo-nitzschia* cultures and genotyped the plankton (or cell)-associated bacteria using 16S amplicon sequencing.
Single-cell isolates of *P. pungens* and *P. multiseries* were obtained from two sites in NB: the NB Long-term Plankton Time Series (NBPTS) and Whale Rock. To identify the microbiomes of our DA-producing (DA+) and non-DA-producing (DA-) cultures, we targeted the 16S rRNA gene and assigned taxonomy using lab pipelines. In preparation for further work, we ran growth curves in triplicate on the cultures to identify the timing of late exponential growth for DA+ and DA- P. multiseries and *P. pungens* cultures. With this information, we will be able to characterize the microbiomes of these cultures and compare them with recent isolates and field samples from NB. Understanding the microbiome of toxic *Pseudo-nitzschia* species in NB is critical for public health protection as it is one of the many environmental factors that affect DA production.

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