Angel Bui

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McNair Assignment

Final Research Paper:

"Niche Partitioning and utilization of different nitrogen sources by marine cyanobacteria *Synechococcus*"

Abstract

The cyanobacterium *Synechococcus* plays a major role in the oceans biochemical processes and is responsible for a significant amount of primary production, especially around coastal areas. *Synechococcus* has a wide geographical distribution that includes both polar and high-nutrient waters. Within the genus there are defined subpopulations that are ecologically distinct that allow them to niche partition the dynamic oceans. To further explore niche partitioning of *Synechococcus*, this project combines a bioinformatic and culture-based approached. Data along the North Pacific Subtropical Front (NSPF) community structure was analyzed. This analysis demonstrated that a particular ecotype identified as clade II (WH 8109) dominated the warmer, nutrient poor waters of the NSPF and was virtually absent in northern, cooler, nutrient-rich areas. This led to the hypothesis that clade II prefers warmer temperatures and nutrient-poor environments. To test this in the lab, representative cultures of clade II were grown in different media that contained variations of nitrogen and nutrient concentrations to reveal their response in growth patterns. These results will further explain the utilization of nitrogen sources and how ecotypes of *Synechococcus* are partitioned among related ecotypes.

The marine picocyanobacteria Prochlorococcus and Synechococcus are the most abundant and widespread photosynthetic cells. Synechococcus is extensively diverse with more than 20 genetically distinct clades identifies, yet their physiology and biogeography are not as well-known as those of Prochlorococcus. Each genus contains subpopulations with physiological difference that could allow them to occupy different environments (Sohm et al, 2016). These picocyanobacteria are major contributors to the biogeochemical cycles on earth, carrying out about 25% of the total carbon fixation in the ocean (Kim 2018). Investigating their distribution characteristics would provide valuable information on future predictions of oceanic conditions, weathers patters, and atmospheric conditions.

Synechococcus are the only bacteria known to perform oxygenic photosynthesis, such crucial roles would inevitably be affected by global warming and result in changes of marine microbial communities. They are the major primary producers of the surface waters with a wide geographical distribution ranging from temperate to tropical regions. Where the picocyanobacteria coexist in the open ocean, Prochlorococcus are more abundant than Synechococcus, although Synechococcus can be seasonally dominant species during blooms. Their slightly larger cell size (0.6-2mM) allows them to fix an order of magnitude more than its counterpart. Synechococcus in stratified oceanic conditions are accompanied by abundant populations of Prochlorococcus, these two genera are closely related but is distinguished by different photosynthetic apparatuses. Prochlorococcus is laterally limited to 40S-40N

but can occupy the whole photic zone, in contrast to Synechococcus, which is restricted to the upper half of the euphotic zone (Partensky et al. 1999).

The versatility of marine Synechococcus has been related to its ability to grow over a wide range of light intensities and spectral quality. Different isolates have been reported to utilize NH_4^+, NO_3^-, NO_2^-, urea, and amino acids as the sole N source while employing systems for active uptake of these compounds (Moore 2002). This work addresses environmental variables that drive patterns of Synechococcus diversity. Previous studies have shown strong niche partitioning at the sub-ecotype level across oceanographic gradients such as temperature, nutrients, and light availability. This fine scale partitioning is not well understood and is further investigated in this study through high-throughput, cyanobacterial-specific, sequencing of high-resolution depth profiles from three stations across the North Subtropical Front (NSPF).

Preliminary data from the SMILE cruise in March 2017 shows specific sub-ecotypes dominating certain latitudes and depths. Through analyzing Synechococcus ecotype community structure changes across SMILE gradients, Synechococcus ecotype WH8109 (Clade II) seems to be dominant sub-ecotype in warmer, tropical waters at 26ºN but then nearly nonexistent in cooler waters of 30ºN and 35ºN. These observations have led to the hypothesis that Synechococcus ecotype Clade II partitions for warmer temperatures and grow better in nutrient-depleted waters, compared to the cooler, nutrient-rich waters further north. In order to better understand the reason behind the apparent differences and determine where there are N utilization differences among members of these genera, this study aims to test the physiological potential of N utilization between two Synechococcus strains that express opposite distribution characteristics.

Methods

Two ecotypes of Synechococcus were examined in this study. Synechococcus ecotypes WH8109 (Clade II) was obtained from Sargasso Sea GenBank and GEYO81156 (crd1) is from the Costa Rica Dome. Both ecotypes are non-axenic, and cultures were obtained courtesy of N.A. Ahlgren, Clark University, Worchester, MA culture collection. Stock cultures were maintained at 24ºC on a light: dark (LD) cycle in a 50/50 L1 and Pro99 media. Both mediums began with artificial seawater (AMP1): 1mL of 1M HEPES free acid buffer(pH7.5), 6 mL $NaHCO_3$ buffer, and Turks Island Salt Mix. AMP1/L1 medium was prepared with: 1L AMP1, 1mL NaH_2PO_4-H_2O, 1mL $NaNO_3$, 1mL SiO_3, 1m trace element solution, and 0.5mL vitamin solution. AMP1/Pro99 was prepared with: 1L AMP1, 1mL NaH_2PO_4-H_2O, 1mL NH_4Cl, and 1mL PRO99 trace metal mix. All nutrients were sterilized by filtration to avoid any chemical change from autoclave sterilization, then added aseptically to autoclave sterilized AMP1 artificial seawater. Detailed media concentrations can be found at the National Center for Marine Algae & Microbiota website.

To test each ecotypes growth on different N sources, AMP1/L1 experimental media were made in which 500 µM NO_3^- was replaced with 50µM NH_4^+. Phosphorous content was also reduced to 10µM PO_4^{3-} so that the resulting media would have a N: P ratio of 5:1, ensuring that when the cultures reached saturated growth phase, N would be growth limiting (Moore et al.). In all experiments, there was a no-N control medium that served as a reference point for different trials. Growth in the no-N cultures reflected the carryover of N from the standard growth condition and all cultures were grown with replicates of 3 with 30 mL of AMP1-L1/PRO99 media and 1mL stock culture in 50 mL glass test tubes. Growth response was determined by measuring culture densities at the same time each day for a duration of 14 days.

A FAC-Scan flow cytometer is used to enumerate cells and a fluorometer was used to measure bulk fluorescence. The reason for FAC-Scan flow cytometer is to ensure that the changes in bulk fluorescence were due to growth in cell numbers and not to changes in phycobilisome fluorescence per cell. After the experiment has concluded, excel is used to plot of growth curve of each ecotype to show the change in relative fluorescence over time. Samples taken are then ran through the FAC-Scan flow cytometer for readings of cells per milliliter. This data set is then plotted over time and the two graphs further analyzed.

Results

Discussion

Works Cited

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