

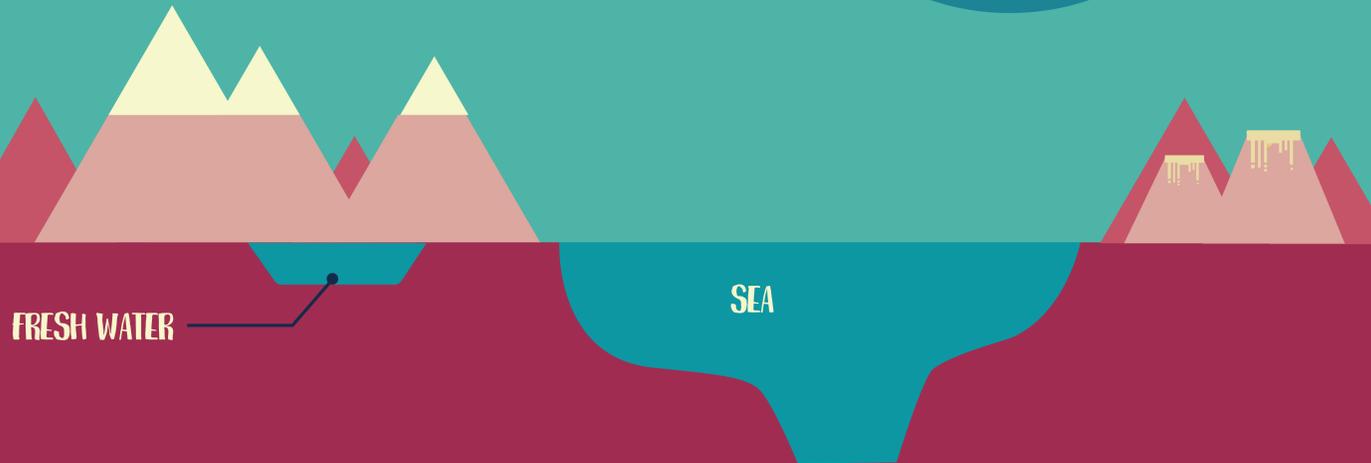
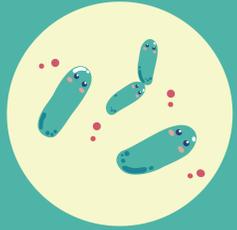
# Culturing of SAR 11 Bacteria

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# SAR 11 ACCOUNT FOR UP TO 30% OF THE OCEAN'S PHYTOPLANKTON!



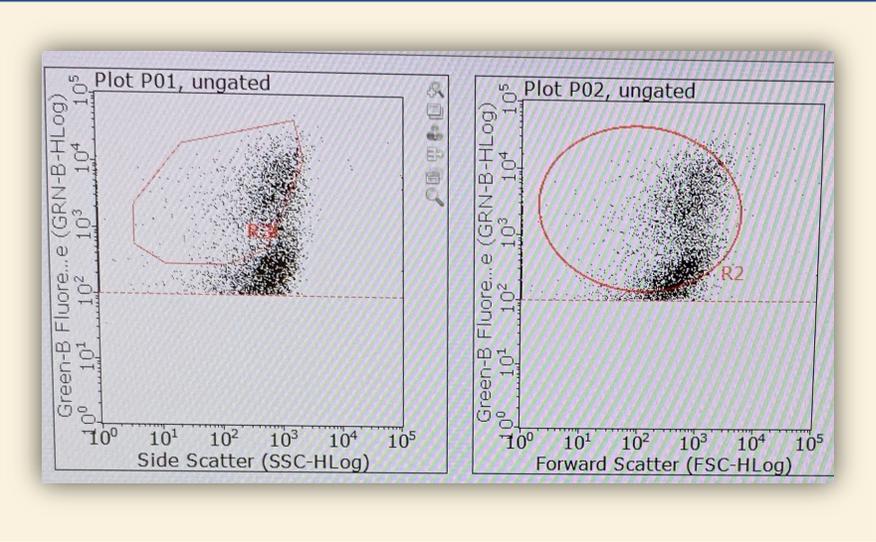
THIS MAKES THEM THE MOST PLENTIFUL ORGANISM ON EARTH

- Genome is minimal (about 1.3 million base pairs), smallest of any known free-living cell.
- SAR11 clade is very diverse, is found in up to 300 meters deep in every body of water.
- SAR11 has enormous contributions to ocean carbon cycling.
- They live in extreme oligotrophic conditions on minimal nutrition.



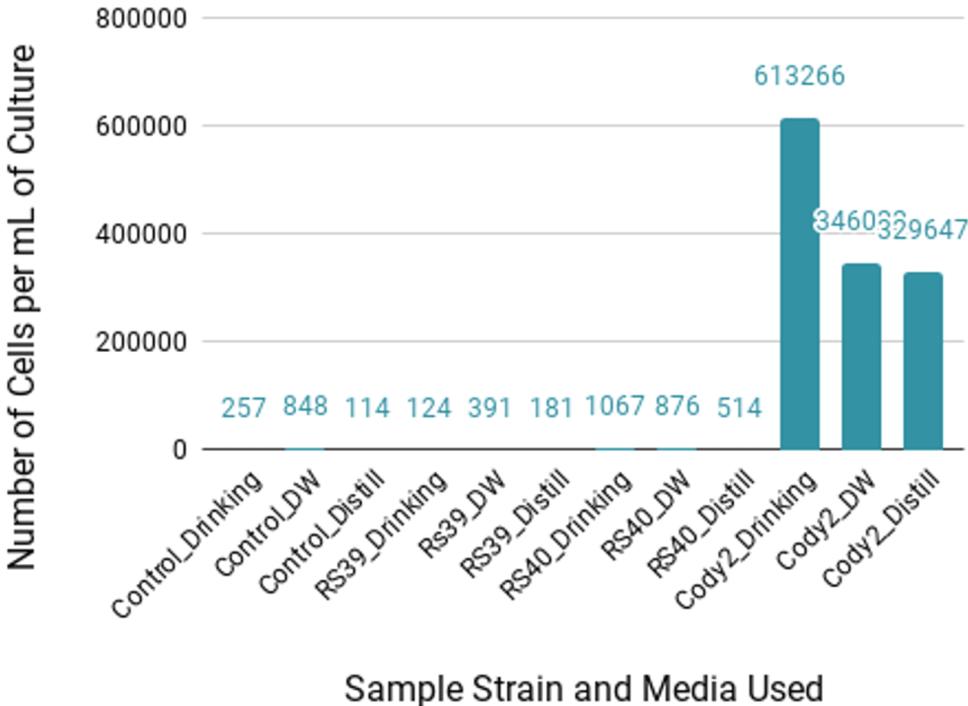
# Flow cytometry results from the 35th day of inoculation.

## Side Scatter and Forward Scatter of Cody 2



Each dot represents a single cell analyzed by the flow cytometer. The characteristic position of different cell populations is determined by differences in cell size and granularity.

### Cells per mL on 35th Day After Inoculation of Cells into Media



## Gel Results of PCR after DNA Extraction

L      C      +      -



# Conclusions

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- All Cody2 cultures were combined to extract DNA.
- A PCR was performed to verify DNA is Cody2.
- If positive, the extracted DNA would be sent for laboratory genomic sequencing.
- Unfortunately, my first gel had a negative result.
- I would do a PCR again to verify it is indeed negative.

# Cultivation of SAR 11 Bacterium: The most abundant organism on Earth.

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Miami Dade College North Campus Miami, Fl and University of Florida Dave Extension



## Introduction

The SAR 11 clade is possibly the most abundant organism on earth. This extreme oligotroph makes up one-third of the bacteria in the ocean. This ancient clade has evolved to be very small, slow-growing, and requiring minimal nutrition. Its abundance indicates vast contributions to the marine ecosystems. Despite copious amounts of SAR 11 in nature, it is challenging to culture in the lab, leading to limited studies on their physiology, including analyses on their potential for adaptation to changes in environmental conditions (e.g., ocean acidification, increase in nutrient concentrations). The SAR11 clade consists of several subgroups that differ in abundance based on environmental conditions like temperature, water depth, and nutrient conditions. Learning more about the organism would help better understand the ocean's ecosystem and health.

## Purpose

Nearly all of the studies on the physiology of SAR11 have been done with a singular strain, representing a single ecotype (subgroup Ia) that is abundant in surface waters of open ocean habitats. To better understand its contributions to the ecosystem, testing of other strains is necessary. In this study, we want to test an artificial media to grow a novel SAR11 strain of subgroup III, Cody2, isolated from Florida Bay. Due to its unique nutrient requirements, SAR11 cultures neither grow on agar plates nor produce densities high enough to see visible changes in optical density (OD) in the media; therefore, growth has to be monitored with flow cytometry. Here, we will present data on the growth of Cody2, illustrate our method to use flow cytometry for measuring bacterial cell numbers, and report on the phylogeny of the novel strain.

## Methods

Cultures inoculated on December 24, 2019 were checked via flow cytometry for cell counts using Gauva ViaCount Reagent Assay on a weekly basis to monitor growth. Cultures were inoculated by taking 500 uL of culture and 50 mL of media. Media was prepared the same except three different sources of water where used to test which worked best; DW- Davie West, Distill – Dionized Water Water, and Drinking Water –Tap media were all prepared with one flask of just media for control, one flask Red Sea Strain 39, one flask Red Sea Strain 40, and Cody 2 coastal Florida. Flow cytometry assays were conducted by taking 198 uL of culture and incubating with 2 uL of Sybr green 15 minutes for staining after cleaning, and calibration of the flow cytometer counts are performed. Attempts to revive Red Sea strains 39 and 40 by inoculation were made on February 5, 2020. Cody 2 samples inoculated on Feb. 20, 2020, to collect more BioMass for DNA...

## Methods cont..

extraction. All inoculations were prepared under sterile, and UV treated conditions using auto clade sterilized glassware. Cultures were kept at room temperature in the dark

DNA extractions of Cody 2 samples made to confirm growth are indeed SAR11 on February 5, 2020, using Ultra Clean® Microbial DNA Isolation Kit by MO BIO Laboratories, Inc. Extracted DNA was run through PCR amplification using Go Taq PCR Buffer and Primer 27F, Primer 2 1492R, dd H20 (DNase, RNase-free) and r 33 as a positive control. PCR produced positive results, but not enough DNA was produced to send for laboratory sequencing. A second DNA extraction was performed using all Cody 2 cultures and a different method of extraction, DNeasy power water kit. All extractions where stored in freezer at 4°C.

## Results

Sample ID	Actual (# cells/mL)
Control_Drinking	257
Control_DW	848
Control_Distill	114
RS39_Drinking	124
RS39_DW	391
RS39_Distill	181
RS40_Drinking	1067
RS40_DW	876
RS40_Distill	514
Cody2_Drinking	613266
Cody2_DW	346022
Cody2_Distill	329647

Figure 1 Cell counts of controls and cultures.

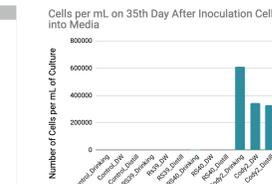
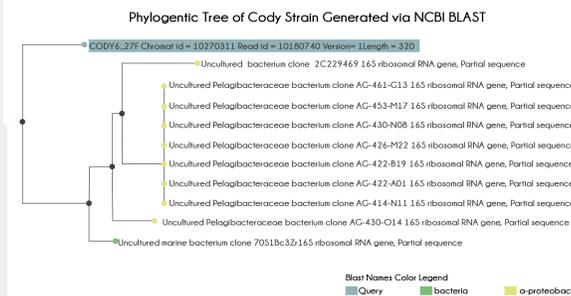


Figure 2 Graph of Cell counts



## Discussion

SAR11 live in varying ecotypes and the nutrient requirements are mostly unknown. The Red Sea strains likely have different nutrient needs. The SAR 11 clade is notorious for being slow growing and relatively dormant, which is a possible they need more time to grow. (Giovannoni,2017). Growing SAR11 in its many ecotypes is necessary to learn more about ocean cycles in carbon and nitrogen fixation. Once proper media has been established more studies can be done to understand this mysterious organism.

## Conclusion

The Cody 2 strain grew in the media. Reviving the Red Sea Strains did not appear to work. Longer incubation times may be necessary and different nutrient content of media. Despite combining all of the Cody 2 strain culture minimal DNA was extracted. Larger amounts of culture could remedy this issue. Further optimization for media culture is needed to have enough biomass for proper genome sequencing.

## References

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