# Comparative Analysis of SAR116 Bacteria and Protein Identification Related to the Assimilatory Sulfate Reduction Pathway

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#### Introduction

- SAR116 = clade of common heterotrophic bacteria
- Role in marine hypoxia (Coehlo et al., n.d.) = occurrence of low oxygen levels in the ocean, often eutrophication, where high levels of nutrients in water from fertilizer run-off (Chislock et al., 2013)
- Excess nutrients → excessive phytoplankton growth → excess dissolved organic carbon and detritus utilized by oxygen-consuming heterotrophic bacteria → increases amount of respiration (Buchan et al., 2014)
- Abundant in Oxygen Minimum Zones (OMZs) with alternative respiratory pathways utilizing an electron acceptor other than oxygen, anaerobic respiration (Lumen Learning, n.d.)
- Alternate electron acceptors is sulfur in dissimilatory sulfate reduction pathway (energy from reducing sulfate and respiration occurs without oxygen (Rabus et al., 2015))
- Comparative genomics analysis of proteins in pathway to observe unique SAR116 adaptations that better suits them for survival under hypoxic conditions and potential major role in marine hypoxia
- Observed proteins in assimilatory sulfate reduction pathway using sulfur for biosynthesis
- Enzymes reduce inorganic sulfate into hydrogen sulfide that isolates sulfur to make cysteine
- Key enzymes = sulfate adenylyltransferase, adenylylsulfate kinase, thioredoxin, and sulfite reductase (MetaCyc, n.d.)

## Purpose

- Investigate the annotated proteomes of the three LSU cultivars in order to find enzymes involved in the assimilatory sulfur reduction pathway that were specific to SAR116
- Determine whether the identified enzymes conferred some unique advantage for SAR116 members to thrive off of inorganic sulfur compounds in hypoxic environments

#### Methods

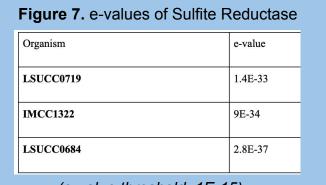
- Genomes of 3 strains of SAR116 bacteria (LSUCC0684, LSUCC0719, LSUCC0744) and others analyzed to identify important proteins unique to SAR116
- With bash version 3.2.57(1) to assess the presence of certain proteins in numerous bacterial strains by running the command dogmatic -prod hmm -i mCURE\_Tools/lab\_02.24.21\_dir/quiz\_5\_data\_dir/ -o mCURE\_Tools/lab\_02.24.21\_dir/prot\_dir -pfam mCURE data/hmm db/pfam full db/pfam-A.hmm -fig pfam full -clear, an annotated file of the genomes of 13 different organisms was organised by different proteins that could be found in
- Proteins related to assimilatory sulfate reduction mapped by the KEGG pathway found and compared between the SAR116 genomes and the other microbe's genomes using their respective e-values (Figure 4).
- The presence of various proteins related to dissimilatory sulfate reduction as outlined by the pathway on KEGG was searched for in all of the organisms (Figure 3)

### Results

Figure 5. e-values of Adenylylsulfate

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Organism	e-value	Organisr
IMCC1322	3.4E-73	LSUCC
LSUCC0684	1.3E-29	
HIMB100	1.3E-61	LSUCC
GCF_002101295.1	2.5E-18	IMCC13
LSUCC0744	1.3E-70	HILL AD 1
IMCC9063	1.6E-22	HIMB1
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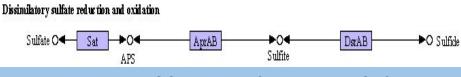
Figure 6. e-values of Phosphoadenosine Phosphosulfate					
Organism	e-value				
LSUCC0744	1.1E-61				
LSUCC0684	2E-65				
IMCC1322	6.4E-63				
HIMB100	1.6E-57				

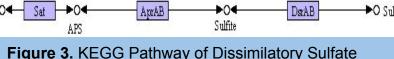


(e-value threshold: 1E-15)

- We identified the presence of adenylylsulfate kinase involved in one of the first steps of assimilatory sulfate reduction (KEGG) in 6/13 strains, 4/6 being part of the SAR116 clade (Figure
- We identified phosphoadenosine phosphosulfate reductase to be in 4 SAR116 strains (Figure 6).
- We identified sulfite reductase, a protein involved in the final step of this pathway of converting sulfite to sulfide (KEGG). We identified the presence of this protein in 3 SAR116 strains (Figure 7).

### Conclusion





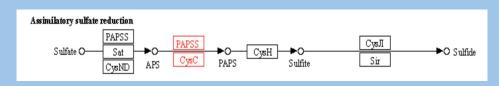


Figure 4. KEGG Pathway of Assimilatory Sulfate

(adenylylsulfate kinase = CysC, Phosphoadenosine Phosphosulfate Reductase = CysH, Sulfite Reductase = Sir)

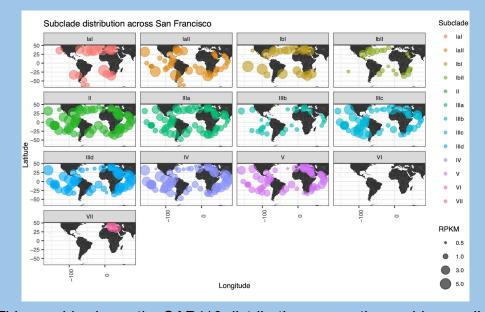
- Proteins related to assimilatory sulfate reduction: significantly present in only SAR 116 genomes
- Sulfate necessary component of biosynthesis, possible alternative proton receptor, obtained and turned into usable form from environment through one of these two pathways
- Assimilatory is better for energy conservation which is important due to anaerobic respiration (Yu et al., 2018)

## **Future Experiments**

- It is still currently unclear why exactly these microbes would rely on assimilatory sulfate reduction rather than dissimilatory sulfate reduction as a means of obtaining sulfate and reducing it.
  - Experiments to better understand the effects of marine hypoxia on organisms living in these zones and the adaptations that they have to survive.
- Compare the two sulfur reduction pathways in various organisms
  - Microbes that have observed proteins involved in dissimilatory sulfate reduction could be observed in hypoxic conditions - the effect of lack of oxygen on sulfur reduction, why organisms living in these zones don't express these proteins.

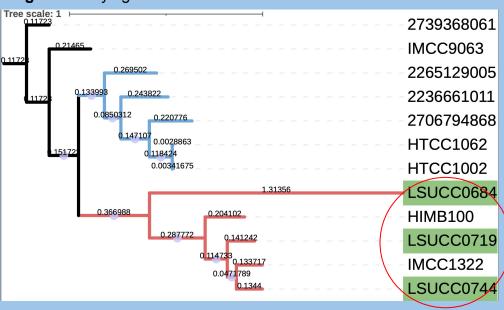
## Supplementary Figures

Figure 1. TARA Subclade Graphic Indicating Distribution of the Various Subclades



This graphic shows the SAR116 distribution across the world, revealing that the dispersal of many of the subclades is relatively cosmopolitan, even abundant in OMZs such as the South Pacific Gyre.

Figure 2. Phylogenetic Tree of SAR11 and SAR116 bacteria strains



The phylogenetic tree indicates the close relationship between the LSUCC strains and HIMB100 and IMCC1322 as part of the SAR116 clade.

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