Reduced Genomes in the Ocean
Specialized Roles in Biogeochemical Cycles

Jim Tripp
KAUST Red Sea Research Center Opening

April 9, 2011
Overview

• The Reduced Genome of SAR11 (*Pelagibacter*)
  - SAR11 clade is most abundant heterotrophic clade in the ocean, one representative is *Pelagibacter*
  - Can genome explain “unculturability”?  
  - Impacts on major nutrient cycles (C, N, P, S)?

• The Reduced Genome of UCYN-A
  - Global nitrogen budget unbalanced
  - N-fixing UCYN-A microbes not yet cultured
  - Can environmental genomics produce a genome?
  - Does the genome give insight into UCYN-A nitrogen fixing efficiency?

• A Slide on “Ground Truth” for Metatranscriptomics
“Sitting through a long-winded speech on nitrogen-fixing symbiotic bacteria, Vance negotiated two deals from his AT&T smartphone.”
SAR11: Ubiquitous, Abundant, Tiny

Morris et al. 2002

Carlson et al. 2009

Nicastro, 2006
SAR11: Why “Unculturtable?”

- Oligotrophic seawater plus ammonium and phosphate works best, but poorly

The more “typical” carbons added, the worse they grow!

Why do they stop at such a low cell density? (<10^6 cells/ml)

Rappé et al., Nature 2002
Genomics: Unique Sulfur Requirement

Environmental fragments containing assimilatory sulfate reduction genes do not have genes with best hits to SAR11 cultured representative.

Metagenomics: Common Requirement

Red = E. coli  Blue = Pelagibacter
Metagenomics: Glycolysis Sometimes Absent!
Can Grow On Acid With Bypass To TCA Cycle

Glyoxylate Bypass Allows Biomass Accumulation.
Unique Control of Glyoxylate Bypass

Pelagibacter growth on organic acids only possible with sufficient glycine.
Metagenomics: Glyoxylate Riboswitch Conserved in SAR11 Populations

Riboswitch fold revealed by intergenic space conservation on metagenomic fragments
Genomics: Abnormal Glycine Metabolism

- Glycine
- Serine
- 3-PGA
- Oxaloacetate
- Glucose

NORMAL

Methyls

Glycine 2C

Serine 3C

3-PGA

SAR11

Glycine betaine

Rare demethylation ($bhmt$)
Essentials: Pyruvate, Glycine, Methionine

Coastal

Open Ocean

Schwalbach, Tripp et al. 2010
Essentials: Pyruvate, Glycine, Methionine

Methionine/DMSP

Glycine
SAR11 Wrap Up

• Unique role in sulfur cycle: reduced S only
• Has largely ceded glycolysis to others
• Specializes in growth on acids
• Special niche role for glycine, methionine
• Speculation: adapted for osmolytes as nutrients?
Current models cannot account for all the nitrogen fixation that is thought to be occurring.
UCYN-A

- Discovered in 1998 with PCR of *nifH*
- Sometimes correlated with high N-fixation
- Can’t grow it in the lab
- Can sort it from seawater
- Can get metagenome from sorted cells
- Lucky break: metagenome $\sim$ genome!
- Does genome give clue to N-fixation rate?
Station 10 dominated by Uncultivated Group A

UCYN-A sometimes most abundant N$_2$ fixer

Zehr, Hewson, Moisander

Station 10 shows high rate of N$_2$ fixation
We Can Sort UCYN-A from Seawater

Brandon Carter, Shellie Bench
Assembly and Closing

GS FLX Paired End Reads (Eight Replicates)

1.42 Mbp Scaffolds (Circularized, But With Gaps)
GS FLX Titanium Reads (Two Replicates)

- Assemble Reads
- Pool Contigs

GS FLX Paired End Reads (Eight Replicates)

- Assemble Reads

Phrap Assembly of Pooled Contigs

1.42 Mbp Scaffold (Circularized, But With Gaps)

- rRNA
- 42 Kbp
- 325 Kbp
- 223 Kbp
- 446 Kbp
- 83 Kbp

Pool Contigs

Scaffold (Circularized, But With Gaps)
Assembly and Closing

GS FLX Titanium Reads (Two Replicates)

Assemble Reads

Pool Contigs

Align Pooled Contigs to Scaffold and Close w/ PCR

1.42 Mbp Scaffold (Circularized, But With Gaps)

Pool Contigs

Assemble Reads

GS FLX Paired End Reads (Eight Replicates)

1.44 Mbp Chromosome

PCR gap 1
PCR gap 2
PCR gap 3
PCR gap 4
Metabolism is Photofermentative

Many biosynthetic pathways absent or incomplete, including carbon fixation, meaning that it is a heterotroph that grows by sugar fermentation.
No O$_2$ Generated by e$^-$ Transport Chain

Could mean much higher nitrogen fixed per cell: no oxygen inhibition of nitrogenase, but still get at least some energy for fixation from photosystem I.
The Lifestyle Paradox

Expect to find it in symbiosis with large partner, e.g. a diatom

Actually find it among tiny, free-living cells

Rachel Foster
UCYN-A Wrap Up

- Photofermentative, symbiotic lifestyle, could mean high $N_2$-fixation
- Genomics support symbiosis
- Wet lab results so far support free living lifestyle, not symbiosis
- Paradox remains for future research
Metatranscriptomics: Ground Truth

Tripp, ms in prep
Metatranscriptomics: Ground Truth

Be careful!
Acknowledgements

Zehr Lab
Kendra Turk, Shellie Bench, Rachel Foster, Brandon Carter, Ian Hewson, Pia Moisander

Giovannioni Lab
Mike Schwalbach, Joshua Kitner, Larry Wilhelm

Collaborators
Brian Desany, Jason Affourtit, 454 Life Sciences
Ronald Breaker, Michelle Meyer, Yale