Harmful Algal Blooms in Utah Lake: A Molecular Insight

Ramesh Goel, Professor
Civil & Environmental Engineering
University of Utah

Participating Students: Hanyan Li, Anwar Alsanea, Carly Hansen; Juhn-Yuan Su, Debolina
Participating Faculty members: Michael Barber; Steve Burian, Sarah Hinners & Brett Clark

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CyanoBlooms is a global problem
Let us take a look at Phytoplankton

Phytoplankton are the autotrophic components of the plankton community and a key part of oceans, seas and freshwater basin ecosystems.

Phytoplankton uses carbon dioxide, releases oxygen, and converts minerals to a form animals can use.

- Float in upper water bodies
- Photosynthesis-autotrophs
- Cyanobacteria and algae
- Eutrophication
  - Algal bloom: non-toxic
  - Cyanobacteria bloom: harmful, be toxic for aquatics and mammals

Mostly cannot be differentiated with naked eyes!
Surface Water Quality: Worldwide issue

Phytoplankton

Excess nutrients (N+P) & mixing

Climate Change

Limiting nutrient for algae growth?

Redfield ratio: 106C:16N:1P
P - limitation?
N - limitation?
Co - limitation?

Conventionally, more P-limited
Now, both N and co-limited more found

Internal vs. external loading?

Internal loading may play a more significant role

Sediment P release
Algae

- **Macro-Algae**
  - Visible to the eye

- **Micro-Algae**
  - Microscopic

**Eukaryotes**

- **Prokaryotes** (Bacteria)

**Cyanobacteria**
- Blue green algae
- Toxic: Microcystin, saxitoxin, anatoxin-a
- Non-toxic: Synechococcus (picoplankton)
- Non-toxin producers

**Chlorophyta**
- Cyanobacteria are a group of oxygenic photoautotrophic Gram negative bacteria

**Toxic**
- Microcystis (Photo: Glenn MacGegor)
- Aphanizomenon (Photo: Grüne Spanalge)
- Anabaena (Photo: Michele Burford)

**Non-Toxic**

**Phytoplankton**

- Cyanobacteria are a group of oxygenic photoautotrophic Gram negative bacteria
CyanoBlooms- A major problem in the US

Source: Graham and others, 2016, USGS OFR 2016-1174)
Cyanobacteria: What are they?

- Ancient group of photosynthetic microbes (3.5 billion years old; Schopf 1993)
- Gram-negative prokaryotic microorganisms
- Originally referred to as blue-green algae
- The only prokaryotes capable of using:
  - Sunlight as their energy (photoautotrophic)
  - Water as an electron donor
  - Air as a source of carbon
  - Air as a source of nitrogen (only for some nitrogen-fixing strains)
Cyanobacteria: Abundance

- First described species was *Phormidium subsalsum*, published in 1829 by Gomont
- Estimates range from 2,000 (Sant’Anna et al. 2006) to 8,000 species (Guiry 2012) with about 150 genera
- 2,698 species identified - 46 of which are cyanotoxin producing (Hitzfeld et al., 2000; Ernstedt al., 2006).
Cyanobacteria-Toxins- fresh and brackish water

- **Microcystin**
  - *Microcystis, Anabaena, Fischerella, Gloeotrichia, Nodularia, Nostoc, Oscillatoria*,
    - Produces microcystins bloom-forming
    - Found in eutrophic lakes,

- **Cylindrospermopsin**
  - *Species that produce cylindrospermopsin: Aphanizomenon flos-aquae, Aphanizomenon qracile, Aphanizomenon ovalisporum, Umezakia natans, Anabaena bergii, Anabaena lapponica, Anabaena planctonica, Lyngbya wollei, Rhaphidiopsis curvata*
    - Hepatotoxin
    - Damages liver and kidneys and possible carcinogen

- **Nodularins: Nodularia Spumigena**

- **Anatoxins: Chrysosporum (Aphanizomenon) ovalisporum, Cuspidothrix, Cylindrospermopsis, Cylindrospermum, Dolichospermum, Microcystis, Oscillatoria, Planktothrix, Anabaena flos-aquae, A. lemmermannii Raphidiopsis mediterranea (strain of Cylindrospermopsis raciborskii)*
  - Neurotoxins (affects central nervous system)

- **Saxitoxins (PSP): Aphanizomenon flos–aquae, Anabaena circinalis, Lyngbya wollei, Planktothrix spp. and a Brazilian isolate of C. raciborskii.**
  - Representative of large family of toxins responsible for Paralytic Shellfish Poisoning (PSP)
  - Deaths recorded to occur 30 minutes after shellfish consumption
Health Impacts of Cyanotoxins

Note: Not all cyanotoxins lead to all of these health impacts. These listed impacts are caused by microcystins or cylindrospermopsin, the two cyanotoxins that EPA has issued Health Advisories for.

**IN HUMANS**

**Brain**
- **Source:** Ingestion
- **Symptoms:**
  - Headache
  - Incoherent speech
  - Drowsiness
  - Loss of coordination

**Respiratory System**
- **Source:** Inhalation
- **Symptoms:**
  - Dry cough
  - Pneumonia
  - Sore throat
  - Shortness of breath
  - Loss of coordination

**Digestive System**
- **Source:** Ingestion, drinking contaminated water, or eating contaminated fish
- **Symptoms:**
  - Abdominal pain
  - Nausea
  - Vomiting
  - Diarrhea
  - Stomach cramps

**Body**
- **Source:** Contact, e.g. swimming
- **Symptoms:**
  - Irritation in eyes, nose, and throat
  - Blistering around the mouth
  - Skin rash, including tingling, burning and numbness
  - Fever
  - Muscle aches (from ingestion)
  - Weakness (from ingestion)

**Organs**
- **Source:** Ingestion
- **Symptoms:**
  - Kidney damage
  - Abnormal kidney function
  - Liver Inflammation

**Nervous System**
- **Source:** Ingestion
- **Symptoms:**
  - Tingling
  - Burning
  - Numbness

**IN PETS**

**Symptoms:**
- Vomiting
- Fatigue
- Shortness of breath
- Difficulty breathing
- Coughing
- Convulsions
- Liver failure
- Respiratory paralysis leading to death
Genes responsible for the production of cyanotoxins

- **Traditional methods**: Detection of cyanotoxins by chemical methods (HPLC, ELISA, LC-MS)
  Cons: detection limit and cyanotoxins can only be detected after release

- **Molecular method**: detection of cyanotoxin-producing genes and gene expression
  Pros: detection of toxin production at early stage, lower than chemical detection limit

**Common cyanotoxin and functional genes**

<table>
<thead>
<tr>
<th>Cyanotoxins</th>
<th>Target organs</th>
<th>Cyanobacteria genera</th>
<th>Functional genes or clusters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcystin</td>
<td>Cyclic peptides, liver</td>
<td><em>Microcystis, Anabaena, Planktothrix, Phormidium</em></td>
<td>mcy genes (two operons, <em>mcyA-C</em> and <em>mcyD-J</em>)</td>
</tr>
<tr>
<td>Cylindrospermopsin</td>
<td>Alkaloids, liver</td>
<td>Filamentous cyanobacteria, <em>Cylindrospermopsis</em>, <em>Aphanizomenon</em></td>
<td>CYN genes (amidino transferase-CyrA, uracil ring formation-CyrG and CyrH, etc)</td>
</tr>
<tr>
<td>Saxitoxin</td>
<td>Alkaloids, Nerve synapse</td>
<td>Many freshwater filamentous, <em>Aphanizomenon</em></td>
<td>sxt gene (31 ORFs, <em>sxtF</em> and <em>sxtM</em> genes, etc)</td>
</tr>
<tr>
<td>Anatoxin-a</td>
<td>Alkaloids, Nerve synapse</td>
<td><em>Anabaena, Oscillatoria</em>, and <em>Aphanizomenon</em></td>
<td>ana gene clusters, anaA, anaB-G</td>
</tr>
<tr>
<td>Anatoxin-a (s)</td>
<td>Alkaloids, Nerve synapse</td>
<td><em>Anabaena, Oscillatoria</em>, and <em>Aphanizomenon</em></td>
<td></td>
</tr>
</tbody>
</table>

Lake systems in Utah

Provo Bay area:
- Algal blooms
- Hypereutrophic in summer
- Considerably higher phosphate concentrations

Farmington bay:
- Wetland sediment pore water
  - Rich in ammonia (>10 mg/L)
  - Phosphate (2 to 7 mg/L)
  - Nitrite (3 to 12 mg/L)

Great Salt Lake, Utah geological survey

Results for July 13, 2017, indicated 104,729 cells/mL of which cyanobacteria represented 77% of the total cell counts.
Introduction about Utah Lake HABs

• Utah Lake experienced harmful algal bloom in recent summers
  (references: DWQ website- Utah Lake HABs)

Limitation of previous study
- Bacterial community during the bloom (microscopy vs molecular )
- The cyanotoxins produced and gene functions are not clarified

Cyanobacterial counts measured in Utah Lake
(source: UDWQ).
Sampling on the Utah Lake

• With the ride offered by DEQ, we have sampled the Lake for three continuous years (16, 17 & 18)

Our Scientific questions

• What is the genetic diversity of cyanobacteria in Utah Lake seasonally?
• How this diversity is related to water quality parameters?
• Are there toxic cyanobacteria?
• What is the ecosystem tipping point?
Different parameters measured

• Typical water quality parameters: pH, nutrients, cBOD, chla, turbidity, temperature

• Genetic diversity: At phylum, genus and species levels

• Toxins: Cyanotoxins using advanced instrumentation

• Functional genes: Using mRNA approach

• P in sediments: P speciation, P release and uptake potential, P sedimentation from water column to sediments.

• Metabolic pathways- connectivity between different bacteria- helping each other- advanced genetic analysis and gene expressions
High-throughput amplicon sequencing - genetic diversity

1. Filter water sample and trap cells onto a filter

2. DNA extraction from environmental sample

3. Amplify DNA markers

4. High-throughput sequencing

5. Bioinformatic processing

6. Ecological analysis

7. Species identification

Plastic-unit 1
Glass-unit 2
Metal-unit 3
High throughput DNA sequencing in 2016-2018

4 sites in 2016, 7 in 2017 and 2018

We are still processing 2018 data
**2016 data**

**Phylum level**

**A** Lindon Marina

**B** Mouth of Goshen Bay

**C** Mouth of Provo Bay

**D** Saratoga Spring

**Cyanobacteria genus level**

**E** Lindon Marina

**F** Mouth of Goshen Bay

**G** Mouth of Provo Bay

**H** Saratoga Spring

Legend:
- Actinobacteria
- Bacteroidetes
- Cyanobacteria
- Firmicutes
- Planctomycetes
- Proteobacteria
- Verrucomicrobia
- Others

Colors:
- Cyanobacterium
- Cyanobium
- Microcystis
- Synechococcus
- Aphanizomenon
- Dolichospermum
- Arthrospira
- Planktothrix
- Others
Cyanobacteria was the dominant phylum in 2017, especially during the bloom season. Actinobacteria and Proteobacteria were the dominant phyla before bloom. It is also noted that Actinobacteria replaced Proteobacteria as the most dominant bacteria phylum at most of the sites in 2017.
Species level in 2016

Synechococcus (up to 77% of total cyanobacteria) is the most abundant picocyanobacteria presented in the lake at non-bloom seasons. Aphanizomenon flos-aquae (up to 88% of total cyanobacteria) was the specie dominated the bloom in both years. More potentially toxin producing cyanobacteria were detected this year.
Comparison between heterotrophs in 2016 and 2017

- **Actinobacteria**
  Corynebacteriales and Micrococcales mainly dominated Actinobacteria community. *Mycobacterium sp.* (order Corynebacteriales) was the dominant genera had a lower relative abundance during the bloom.

- **Bacteroidetes**
  the relative abundance of Flavobacteriales community was highly increased during the harmful bloom of 2016. *Flavobacterium sp.* and some members in Saprospiraceae were able to degrade cyanotoxins or scavenge of cyanobacteria

- **Proteobacteria**
  *Limnohabitans sp.* from the order Burkholderiales was the most dominant OTU observed *Limnohabitans sp.* and Rhodobacterales could live on cyanobacteria exudes
Figure S1. Eukaryotic phytoplankton communities analyzed by chloroplast 16S rRNA gene. (A, B) Relative abundance of chloroplast community at the order level. (C, D) The comparison of sequence reads percent between chloroplast and cyanobacteria communities. Note the differences of y-axis scales.
Genetic diversity- so what

What do they mean among themselves

Important is how they relate to environmental factors

Ecological theory
Network Analysis - how bacteria help each other

Network of co-occurring bacterial genera based on correlation analysis. A connection stands for a strong (Spearman's $\rho > 0.6$) and significant ($P < 0.05$) correlation. The nodes are colored by phylum.

- **Actinobacteria**
- **Bacteroidetes**
- **Cyanobacteria**
- **Proteobacteria**

Ongoing
From the analysis, the growth of cyanobacteria is more correlated with temperature, while heterotrophs were more correlated with bacteria. Different groups dominated different periods of the bloom.
Alpha and beta diversity analysis

• Alpha and beta diversity are ecological indexes, measuring the richness, evenness and vulnerability of an ecosystem.
  • Alpha diversity
    - The diversity of each site, e.g. Shannon, Simpson, chao1
  • Beta diversity
    - The diversity between different sites, e.g. Bray-curtis, UPGMA tree and others
For both 2016 and 2017,

- The alpha diversity and evenness decreased during the bloom.

- The presence of bloom specialists may decrease the evenness of samples.
Figure S2. Beta diversity-UPGMA tree. (A) UPGMA for 2016. (B) UPGMA for 2017. Clusters of samples from MPB are indicated by asterisk.
Figure S3. Three-dimensional Principle coordinate analysis (PCoA) of beta diversity (samples were clustered by month). (A, C) unweighted UniFrac distance matrix for sample comparison in 2016 (top) and 2017 (bottom). (B, D) weighted UniFrac distance matrix for sample comparisons in 2016 (top) and 2017 (bottom). The method is jackknifing analysis in QIIME.
How is cyanotoxin produced and come into human bodies?

Functional gene clusters

Transcription

Formation of mRNA

Translation

Formation of amino acid

Formation of cyanotoxins

Folding of peptides

Bioaccumulation or direct contact
<table>
<thead>
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<th>Oligo name</th>
<th>Sequences (5'-3')</th>
<th>Tm (°C)</th>
<th>Target</th>
<th>bp</th>
<th>Limit of detection (gn rx⁻¹)</th>
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<td>mcyE or ndaFin Cyanobacteria</td>
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<td>mcyG in Microcystis</td>
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<td>mcyEcya</td>
<td>mcyGmis</td>
<td>mcyAmis</td>
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<td>4.03E+03</td>
<td>1.29E+07</td>
<td>3.38E+01</td>
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</tbody>
</table>


*mcyE*, *mcyG* and *mcyA* gene: microcystin production genes, located on the *mcy* gene cluster.

The expression of toxin production gene is less compared with protein synthesis gene.
2018 analysis - some fundamental questions

- Cyano stratification during blooms
- Vertical movement of cyanobacteria - 2018 field data and planned lab tests
- Functional gene network
- What happens to toxins after the bloom and how they affect ecosystem
Acknowledgement: USEPA for providing funding through its STAR funding mechanism. Utah DWQ for helping with sampling and providing a general advice.
Expected analysis for 2018

• Community analysis with temporal and spatial diversity
  - Samples from surface, middle and bottom of the lake were collected

• Function analysis
  - Expression of toxin producing genes
  - metabolic pathways prediction using 16S rRNA genes

• The sediment analysis
  - We have collected samples from the surface of sediment
  - detect the P bounding structures and the P speciation of the lake
Diversity at phylum and Genus level

**Phylum level**

<table>
<thead>
<tr>
<th>Environmental factors</th>
<th>Dominate bacteria phyla</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Actinobacteria</td>
</tr>
<tr>
<td>Temperature</td>
<td>Bacteroidetes</td>
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<tr>
<td>Chlorophylla</td>
<td>Cyanobacteria</td>
</tr>
<tr>
<td>Nitrate</td>
<td>Firmicutes</td>
</tr>
<tr>
<td>Phosphate</td>
<td>Planctomycetes</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>Proteobacteria</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>Verrucomicrobia</td>
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<td>Cyanobacteria</td>
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<td>Firmicutes</td>
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<td>Planctomycetes</td>
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<td>Proteobacteria</td>
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<td>Verrucomicrobia</td>
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</tbody>
</table>

**Environmental factors**

- pH
- Temperature
- Chlorophylla
- Nitrate
- Phosphate
- Actinobacteria
- Bacteroidetes
- Cyanobacteria
- Firmicutes
- Planctomycetes
- Proteobacteria
- Verrucomicrobia

**Dominant bacteria phyla**

- Actinobacteria
- Bacteroidetes
- Cyanobacteria
- Firmicutes
- Planctomycetes
- Proteobacteria
- Verrucomicrobia
<table>
<thead>
<tr>
<th>Pathway</th>
<th>95% Confidence Intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleotide metabolism</td>
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<td>Vibrio cholerae infection</td>
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<td>Cell division</td>
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<td>Germination</td>
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<td>Phenylalanine metabolism</td>
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<td>Streptomycin biosynthesis</td>
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<td>Cellular antigens</td>
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<tr>
<td>Betalain biosynthesis</td>
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</table>
LefSe – visualize the microbial composition differences between two groups.
Specific metabolic pathway comparisons

ko00680 Methane metabolism

ko00196 Photosynthesis antenna proteins
The quantification of mcyD gene copies and expression levels (2017 test)

Gene copies
- Detected at most sites
- Gene copy numbers (#copies/mL) increased since June 15th

<table>
<thead>
<tr>
<th>Site</th>
<th>June 1st</th>
<th>June 15th</th>
<th>July 11th</th>
<th>Aug 3rd</th>
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<tbody>
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<td>7.91E+01</td>
<td>7.45E+04</td>
<td>2.65E+04</td>
</tr>
<tr>
<td>Provo Buoyi</td>
<td>5.93E+00</td>
<td>3.34E+02</td>
<td>2.62E+04</td>
<td>1.51E+04</td>
</tr>
</tbody>
</table>

Gene expression copies
- Not all site has a high gene expression levels
- Toxin expressions were detected in Provo Bay

Mouth of Provo bay

Gene copies (#/mL)

June 15th | July 11th | August 3rd
---|---|---
2.96E-03 | 7.91E+01 | 7.45E+04
2.65E+04