MICROCOLEUS AS TOXIC BENTHIC MATS ON DIFFERENT BOTTOM SUBSTRATES: ECOPHYSIOLOGY AND DISTRIBUTION

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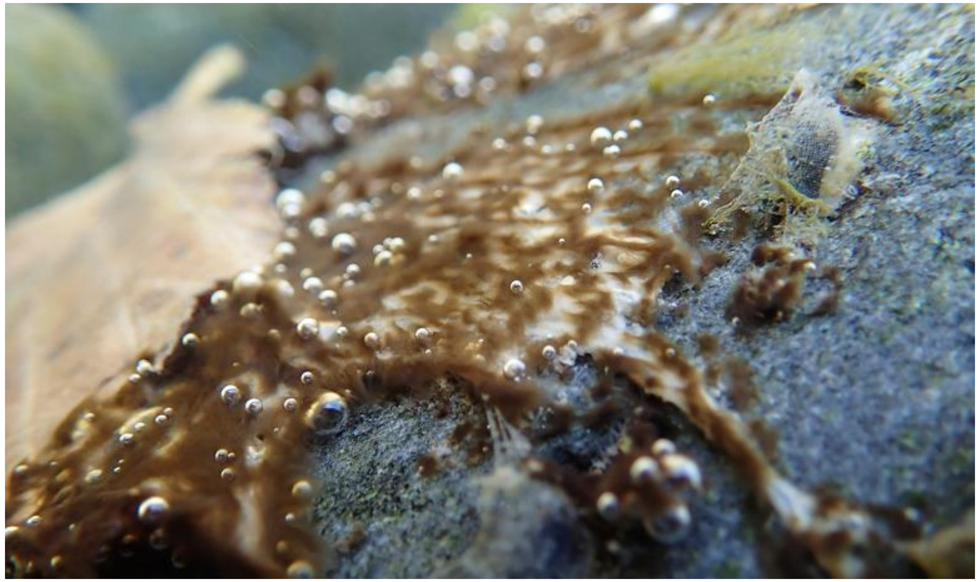
Microcoleus: A Rising Threat in Freshwater Ecosystems

- *Microcoleus* and related cyanobacteria have caused animal fatalities worldwide, with recent cases in the USA, Canada, Europe, and New Zealand.
- The primary cause of these incidents is the ingestion of toxic benthic cyanobacterial mats, often found in rivers, lakes, and streams.
- Fatalities have been recorded since the 1990s, with increasing frequency, posing a significant risk to both animal and human health.
- Our research employs metagenomics to uncover the genetic mechanisms behind *Microcoleus* growth and toxin production. Investigating gene expression helps to understand how *Microcoleus* interacts with other organisms and how these interactions support the proliferation of toxic cyanobacteria.



A *Microcoleus* mat grows over a rock in California's Eel River watershed. Image credit: Keith Bouma-Gregson (University of California, Berkeley) Source: McDermott A, 2024

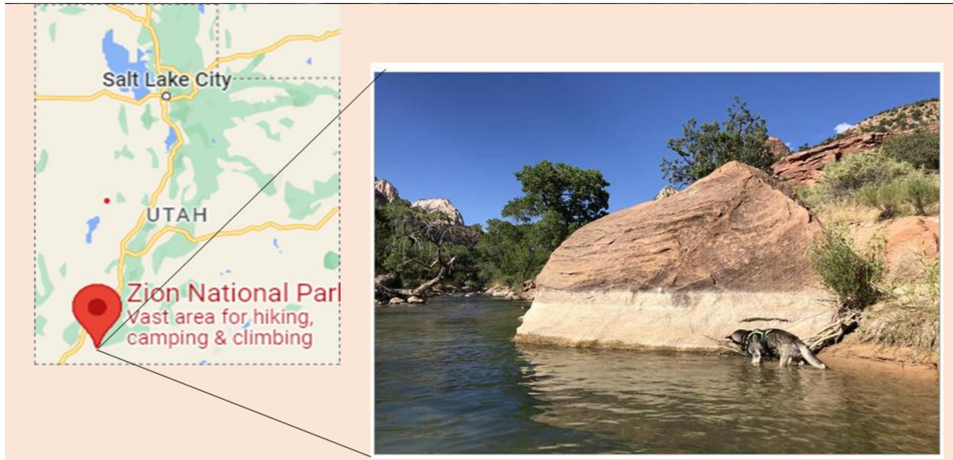
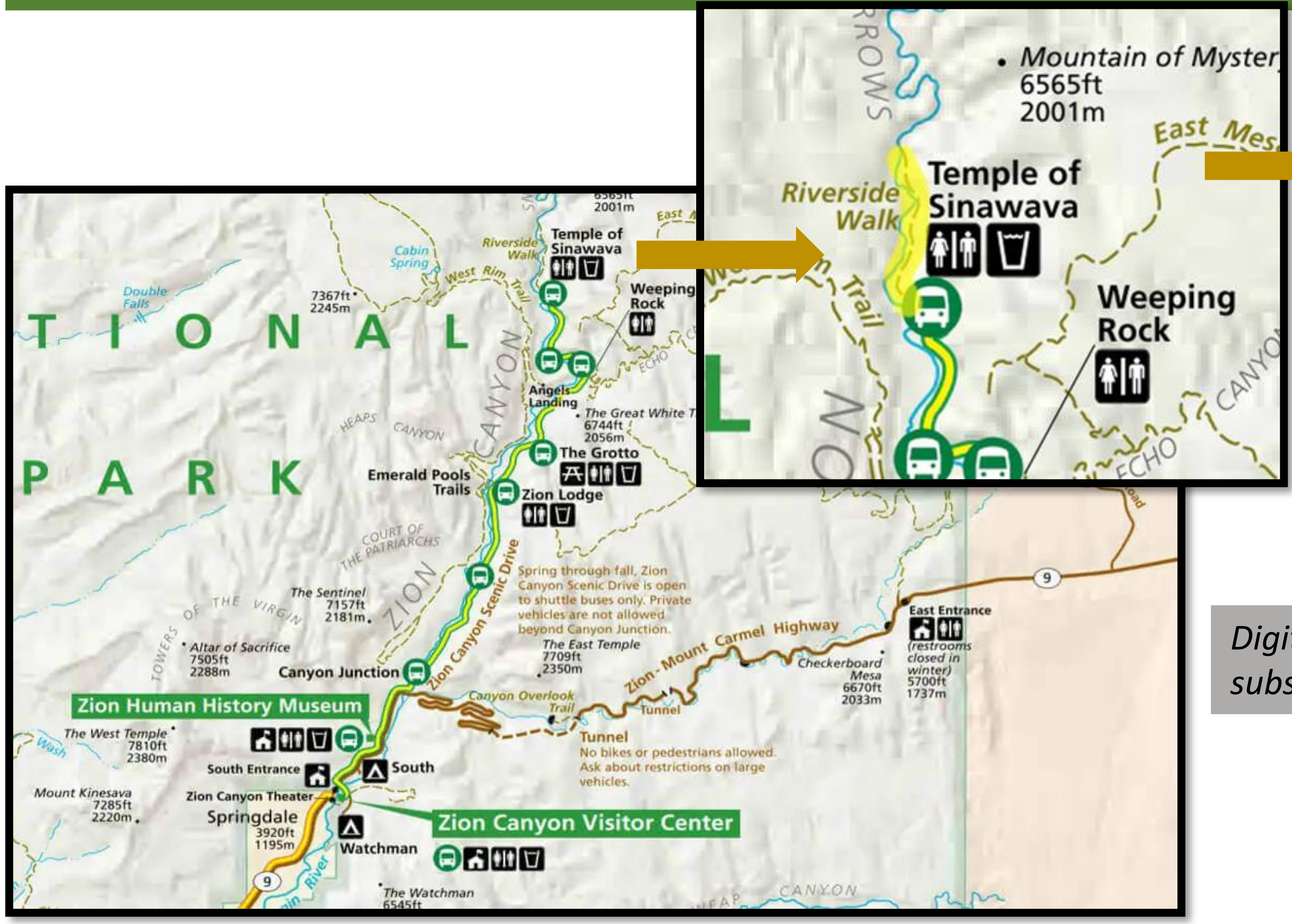
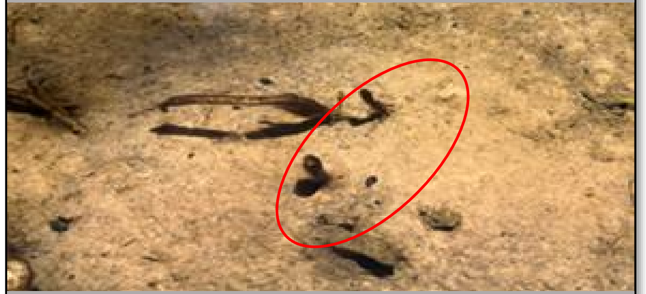


Figure-:A husky was reported to have died after being exposed to algae bloom in the Virgin River(Source: Las Vegas Sun Report on September 14, 2020)

Microcoleus mats at different bottom substrates - Zions National Park



Microcoleus mat growing on a pressure valve near Visitor center, Zions national park



Microcoleus growing on sand substrate near Temple of Sinawava

Microcoleus growing on rock substrate near Temple of Sinawava

Digital Microscopic image of rock substrate

Digital Microscopic image of sand substrate

Sample collection

1. **Benthic Mats**: We collected samples from two substrate types:
   GM: Rock substrate
   SM: Sand substrate
2. **Biofilm Scrapes**: To capture the complete benthic community we scraped the underlying biofilm:
   SS: Biofilm beneath mat on sand surface
   GS: Biofilm beneath mat on rock surface
3. **Water Samples**: Over a 4-hour period, we collected composite water samples for detailed water chemistry analysis.
4. **Sand Samples**: Sand samples were also analyzed for soil nutrition to assess nutrient availability

Sample Collection

Mat Samples:
Rock: GM1, GM2, GM3
Sand: SM1, SM2, SM3

Biofilm Samples:
From rocks: GS1, GS2, GS3
From sand: SS1, SS2, SS3

Analyses Performed

1. **Toxin Analysis**: Composite benthic mats and water from the junction where the tributary meets the Virgin River were sampled for toxin analysis.
2. **DNA/RNA Extraction**: DNA and RNA were extracted from bio-mass samples.
3. **Metagenomic Sequencing**: Illumina shotgun metagenomic sequencing was performed on the extracted DNA and RNA to investigate microbial diversity and functional gene expression.

Toxin Analysis and Water Chemistry

Toxins measured in biomass

Sampling date	Sample Site	Extracted Weight (g)	Microcystin (Detection limits) (µg/g)	Anatoxins (µg/g)	Dihydro Anatoxin-a (µg/g)	Cylindrospermopsins (µg/g)
7th May 2023	Stream Biomass Sample 1	0.2219	nd (<0.19)	389.622	5.328	(<0.02)
7th May 2023	Stream Biomass Sample 2	0.2229	nd (<0.19)	356.439	4.822	(<0.02)
7th oct 2023	Stream Biomass Sample	0.2361	nd (<0.15)	(<0.01)	nd	(<0.01)

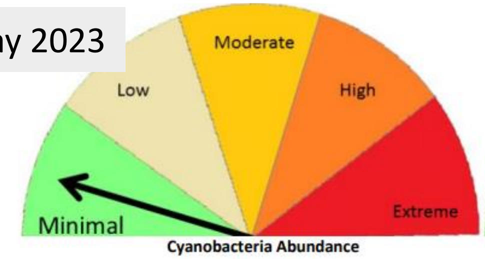
Water chemistry

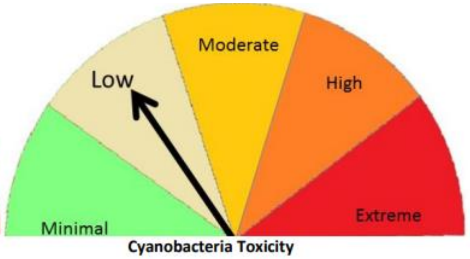
Nitrate-NO3 (Mg/L)	0.265
Dissolved Phosphate (PO4-P mg/L)	Below detecting limit
Total Dissolved nitrogen N (mg/L)	0.3
Total phosphorus (PO4^3- mg/L)	0.12
pH	6.39
DO (mg/L)	8.64
Fluoride (mg/L)	3.4
Chloride (mg/L)	248.69
TOC (mg/L)	1.7

Toxin Levels Measured Downstream at the Junction with the River

Measured at SUNY-ESF Department of Chemistry

7th May 2023

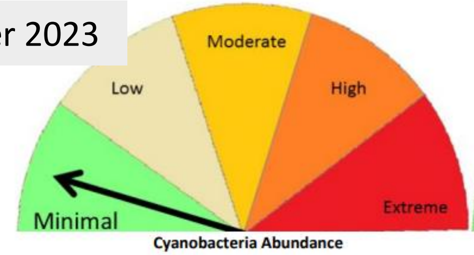


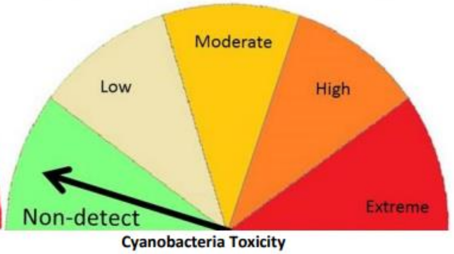


Microcoleus was the most abundant species

ATX(µg/L)= 0.377

7th October 2023





No *Microcoleus* found

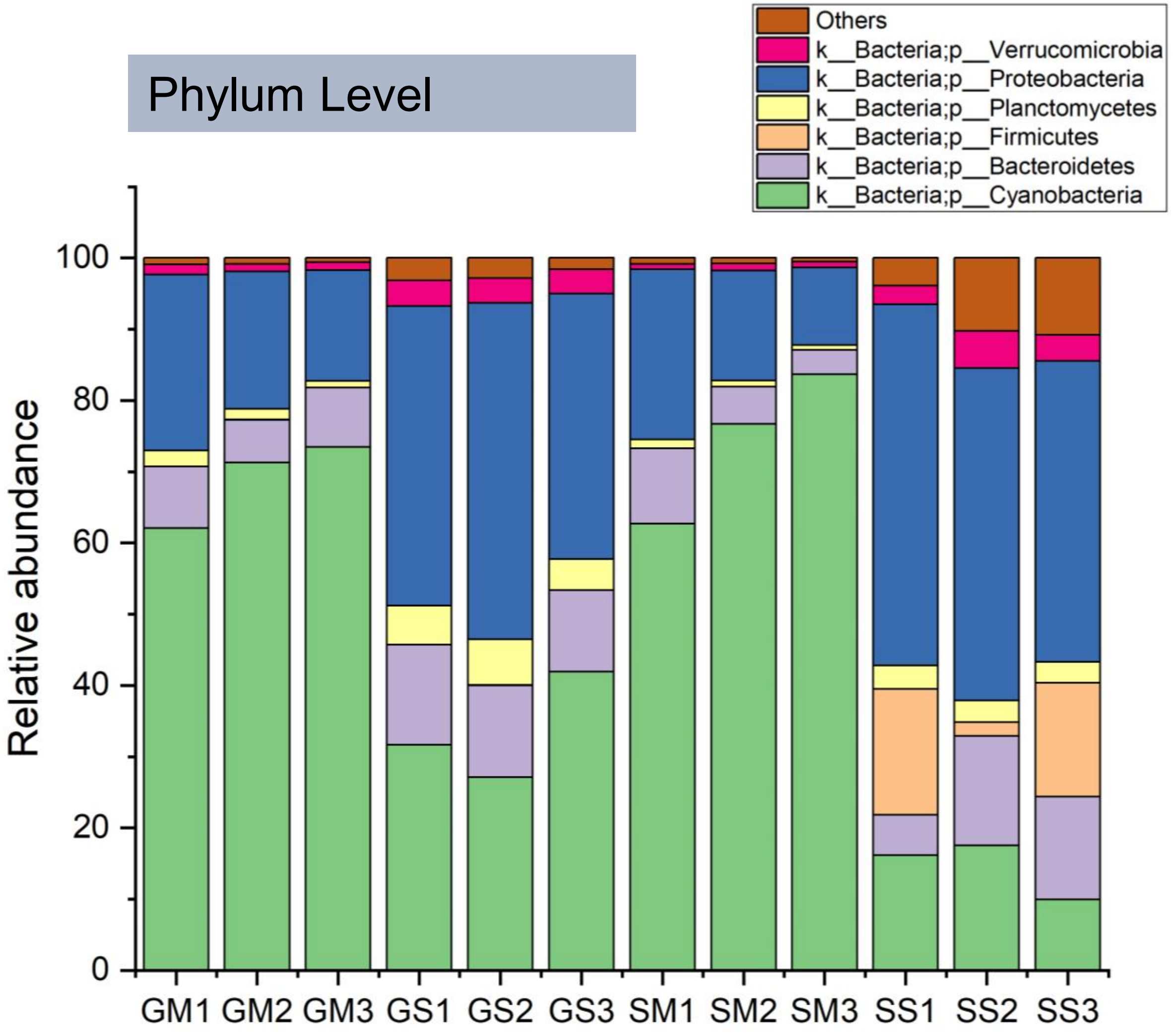
ATX(µg/L)= not detected

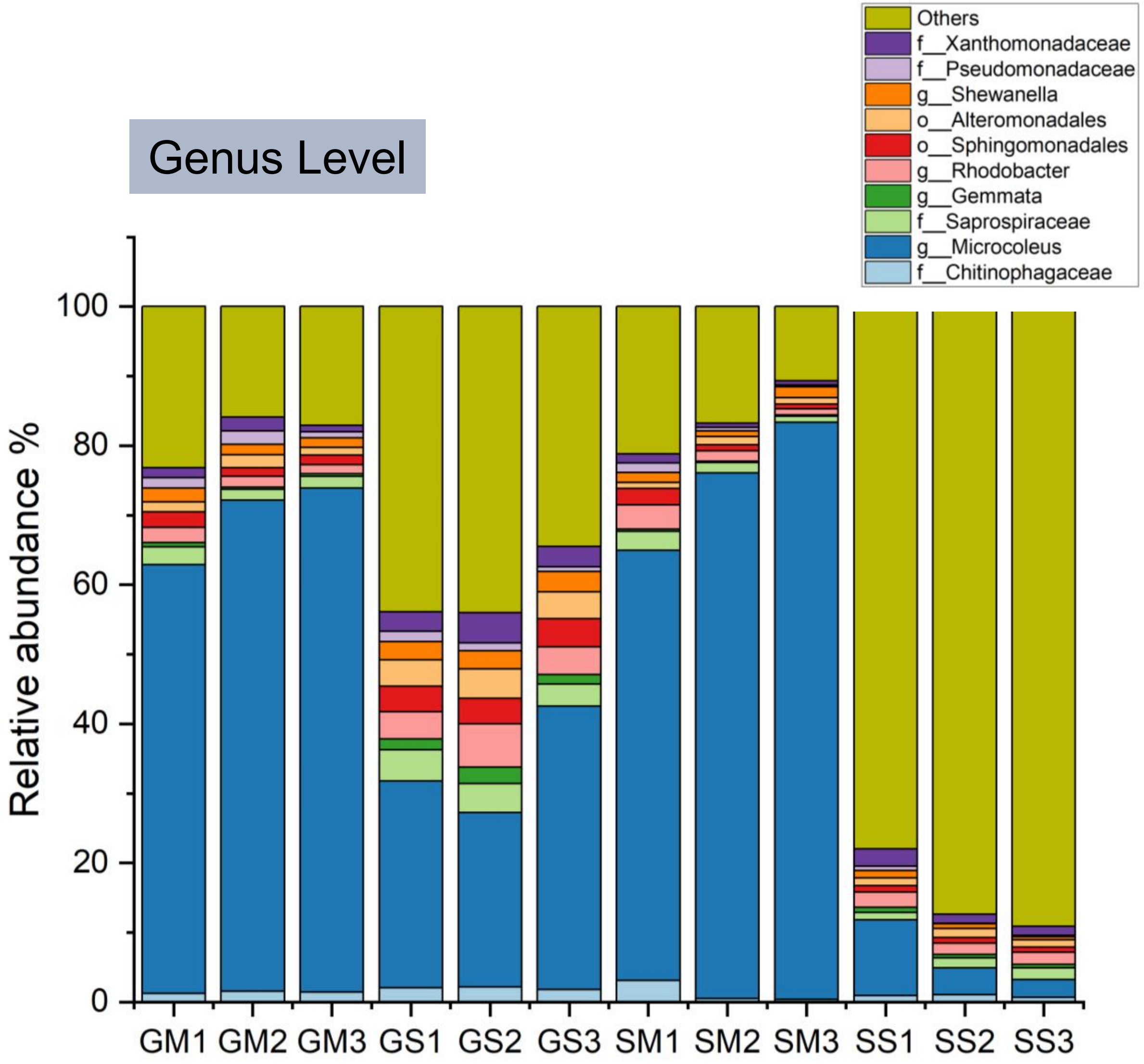
The toxin measured detected from 7th May water samples is likely from some benthic source not from the pelagic algae because of the very low Chl concentration in water column.

Substrate analysis

Nitrate-Nitrogen N mg/kg	SS1	0.46
Phosphorus- P mg/kg	SS1	6.69
Nitrate-Nitrogen N mg/kg	SS2	0.29
Phosphorus- P mg/kg	SS2	4.55
Nitrate-Nitrogen N mg/kg	SS3	2.41
Phosphorus- P mg/kg	SS3	0.08

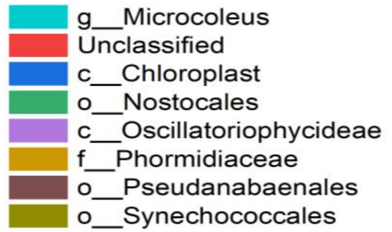
16s rRNA analysis results - Microbial Diversity

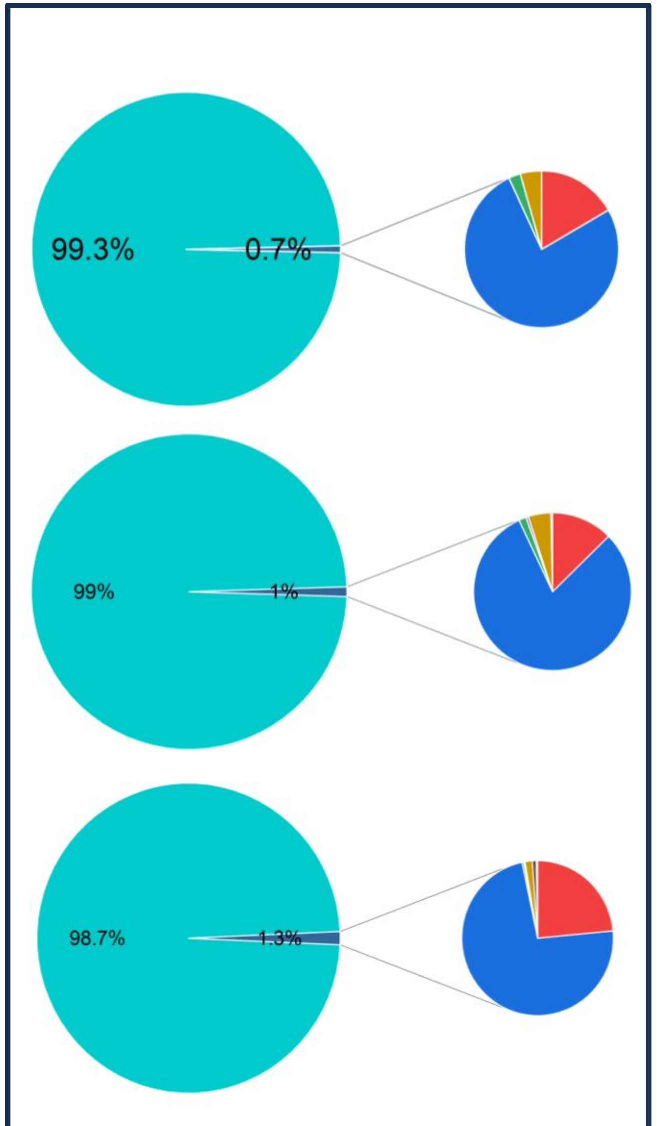




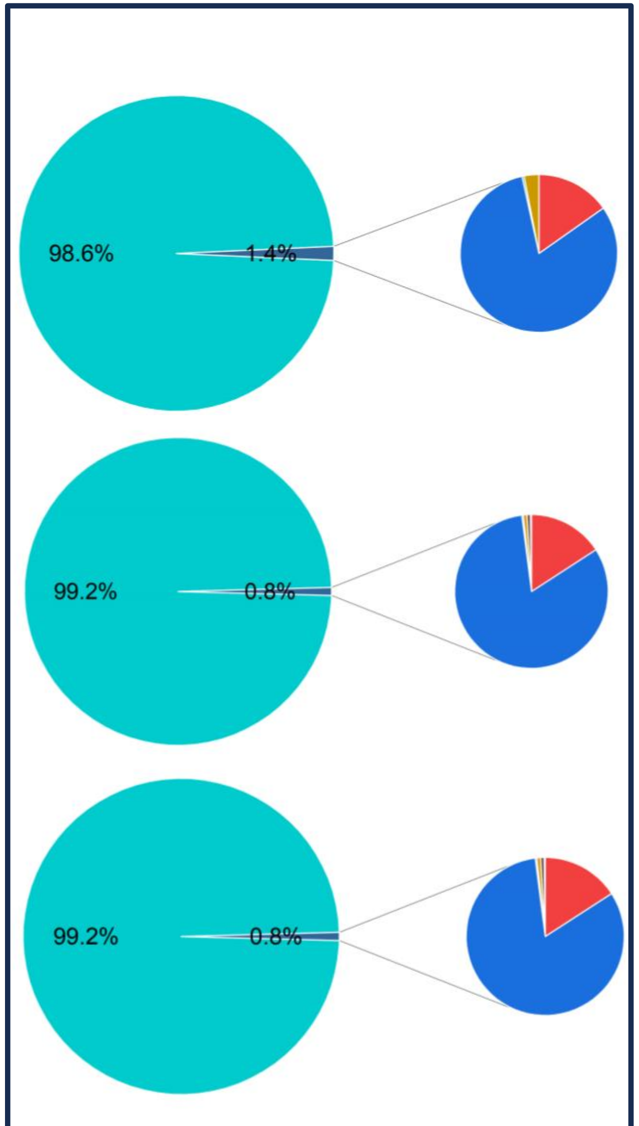
For **7th October** samples**, no *microcoleus*** was found and the most abundant cyanobacteria belonged to class **chloroplast**.

Cyanobacterial Diversity

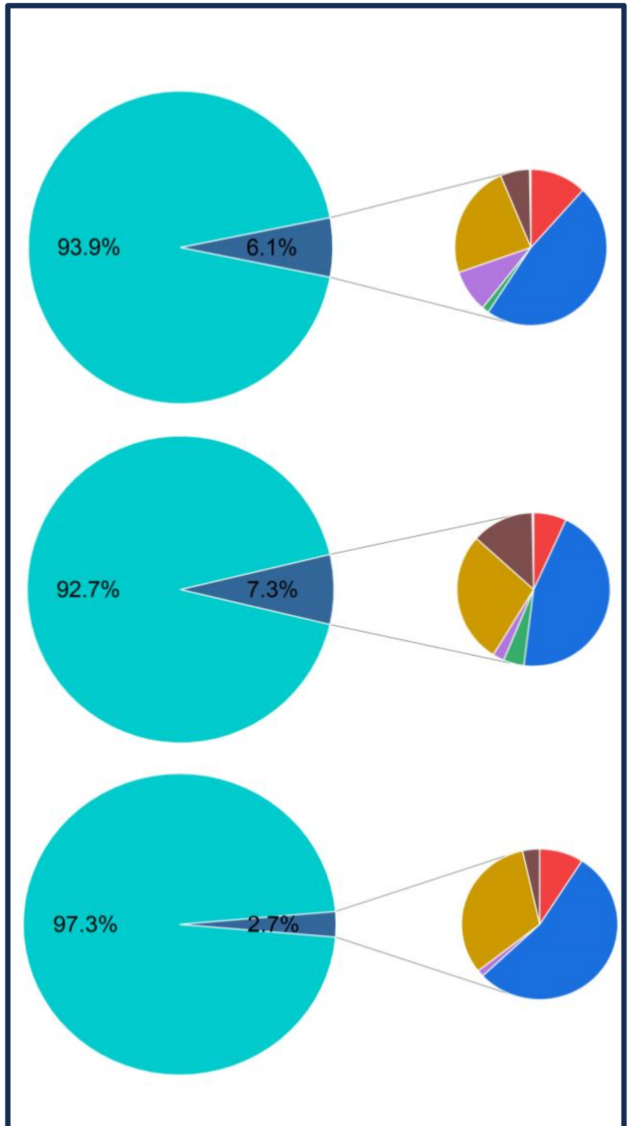




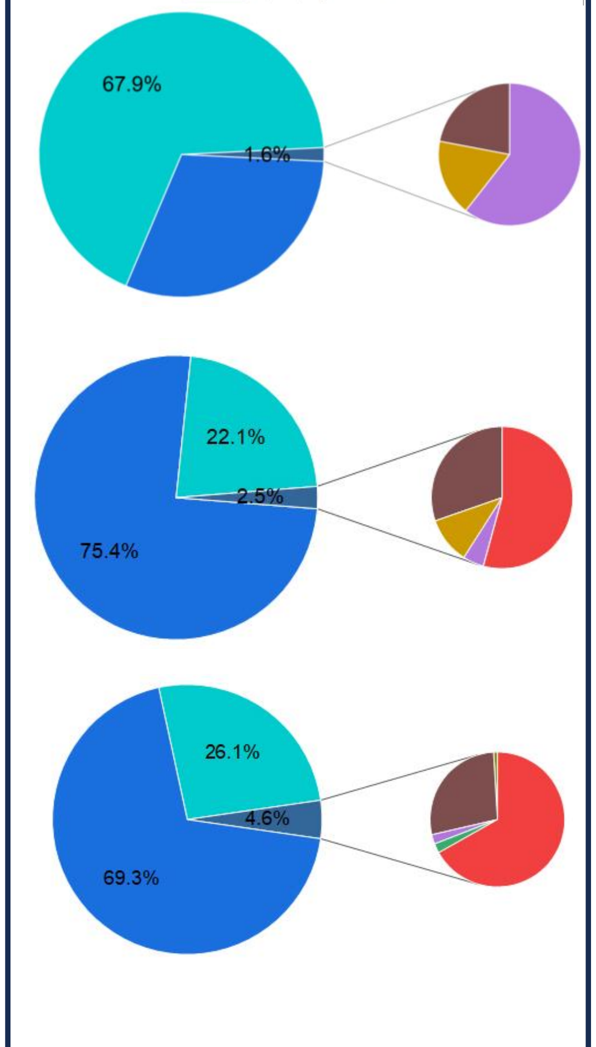
Mats growing on Rock Substrate



Mats growing on Sand Substrate



Biofilm from Rock samples

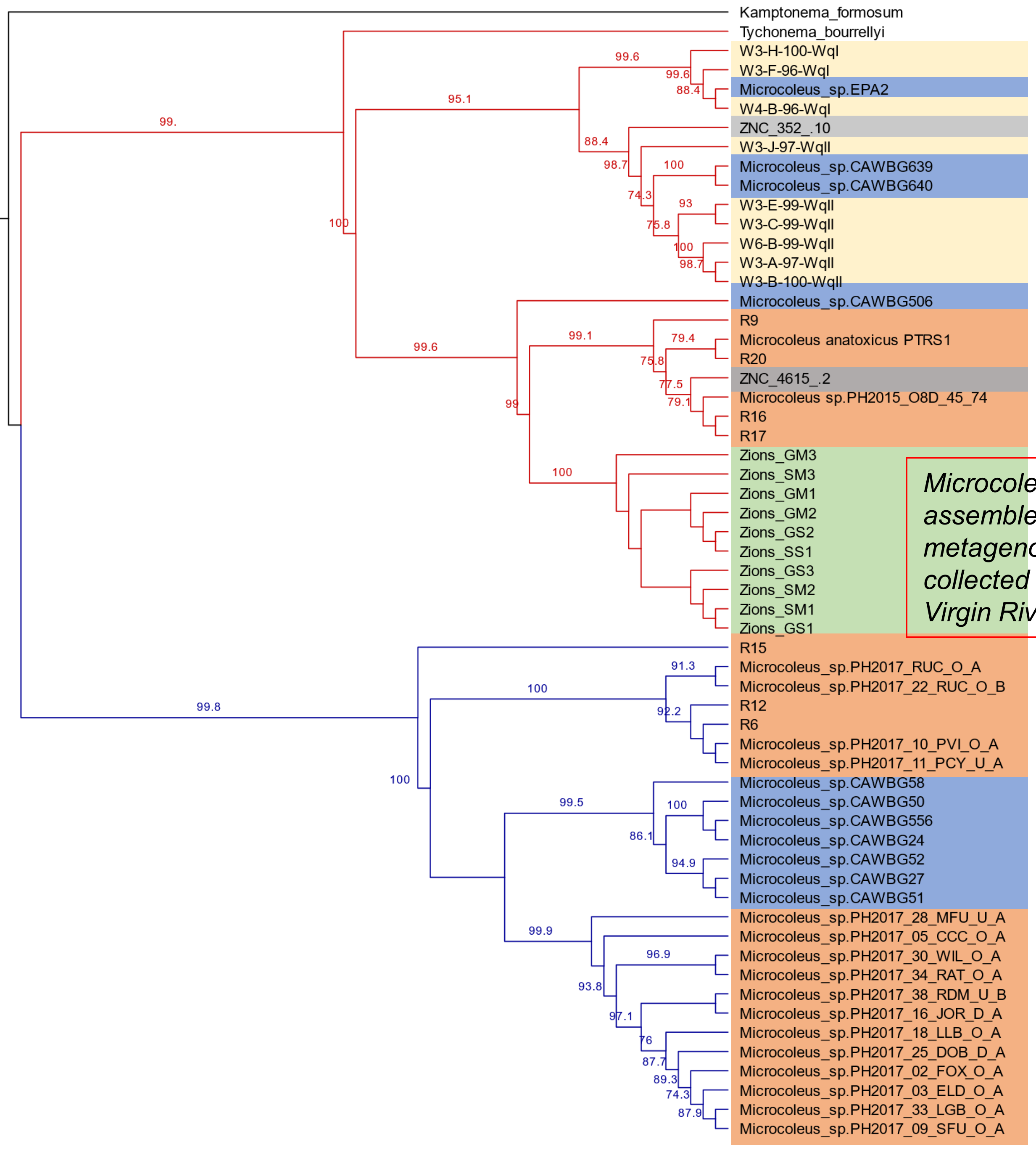


Biofilm from sand sample

Phylogenetic Analysis

Our Microcoleus strains closely related to *Microcoleus anatoxicus* sourced from the Russian River, California, USA

Microcoleus species can be distributed into two clades, toxic (with red branches) and non-toxic (with blue branches)



Microcoleus genomes assembled from the metagenomic samples collected from a side stream, Virgin River

Microcoleus strains from California, USA

Microcoleus strains from New Zealand

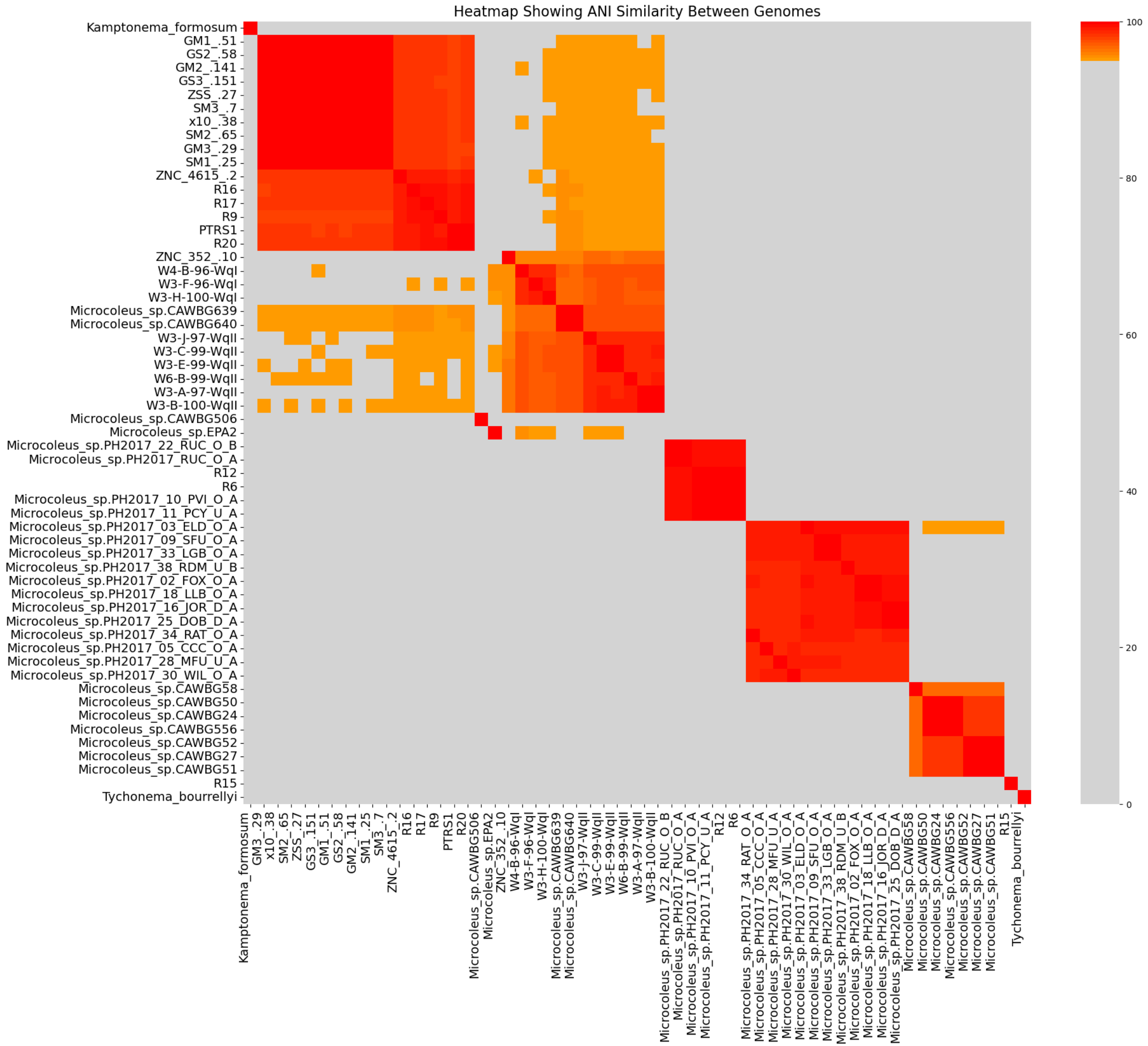
Microcoleus Strains from Canada

Microcoleus Strains from side stream, Virgin River, 2023

Microcoleus strains from virgin river, 2021

Comparison of genomes based on Average Nucleotide Identity (ANI)

Genomes from Zions shares > 98% ANI with *Microcoleus Anatoxicus*

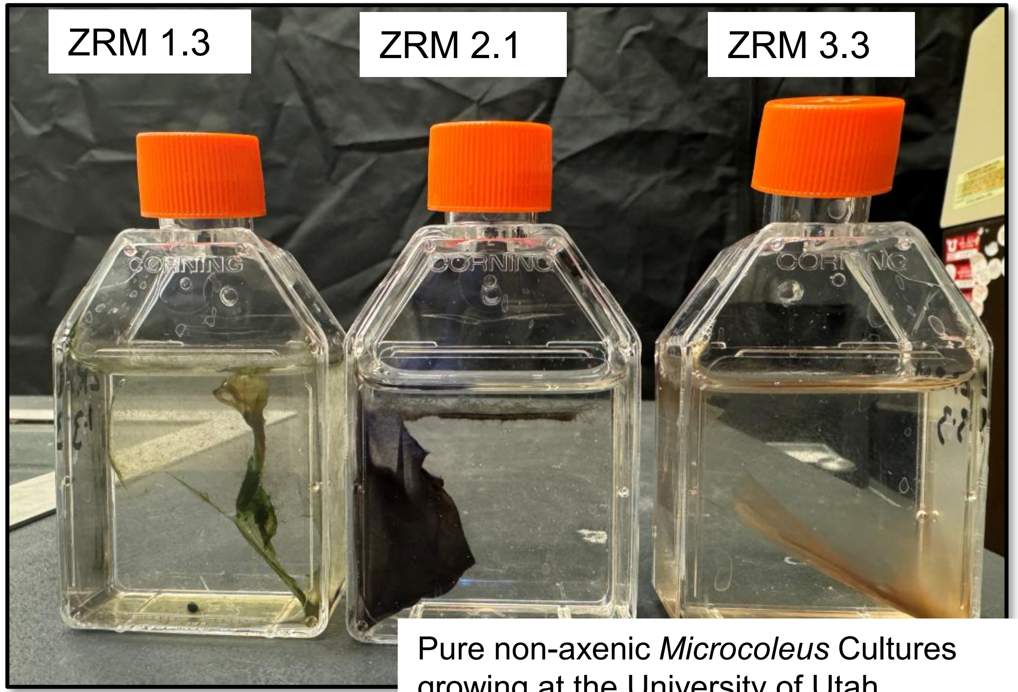


Toxic *Microcoleus* strains isolated from Site

Strain N0.	Sampling Location	Toxin measurements date	Anatoxin-a (ug/L)	Dihydro-Anatoxin-a (ug/L)	Homo-Anatoxin-a (ug/L)	Dihydro-homo-Anatoxin-a (ug/L)	Microcystins (ug/L)	Cylindro-spermopsins (ug/L)
ZRM 2.1	Zion National Park, Small tributary to Virgin River	11/28/2023	11.33	45.39	non-detect	non-detect	nd (<2.07)	nd (<0.19)
ZRM 3.3	Zion National Park, Small tributary to Virgin River	11/28/2023	5.63	34.77	non-detect	non-detect	nd (<2.07)	nd (<0.19)

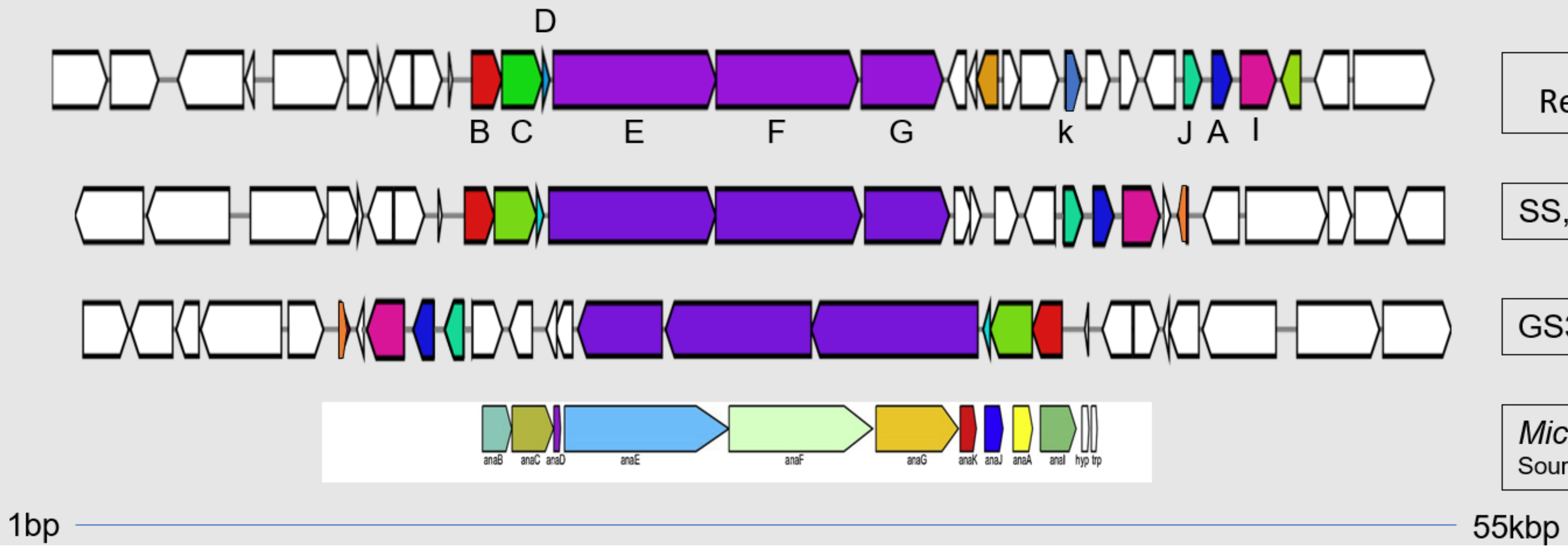
Non-axenic Pure *Microcoleus* strains were isolated at Dr. Rosalina Christova's lab at George Mason University

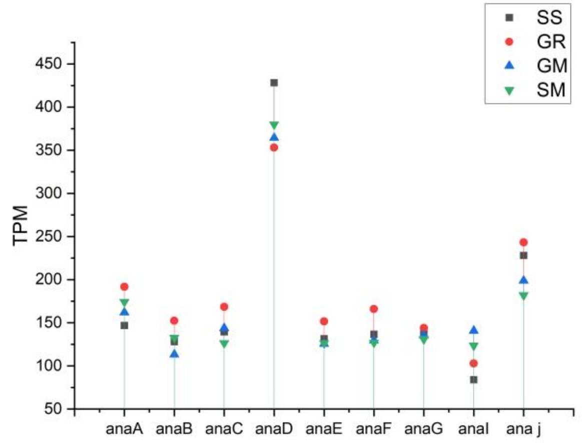
Microcoleus mat collected for Isolation of Pure Cultures at Zion National Park



Pure non-axenic *Microcoleus* Cultures growing at the University of Utah

Anatoxin Gene clusters and Gene expression

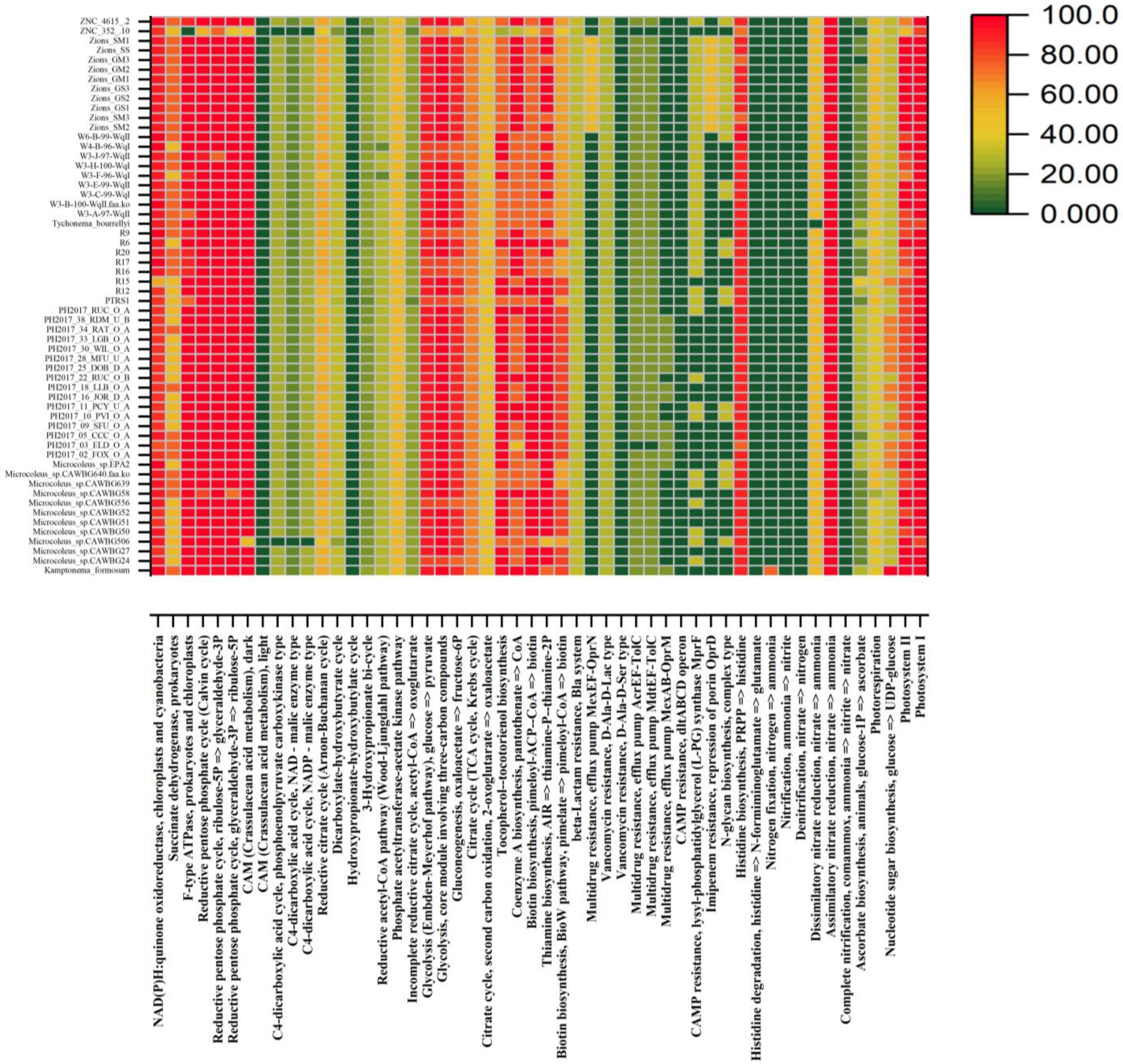




- The complete *Ana* gene cassette responsible for anatoxin biosynthesis was identified across all *Microcoleus* genomes analyzed.
- Interestingly, while the ***AnaK* gene was not located within the T1PKS region, it was still detected elsewhere** in the genome.
- All other genes in the *Ana* cassette, including *AnaB*, *AnaC*, *AnaE*, *AnaF*, *AnaG*, *AnaJ*, *AnaA*, and *AnaI*, demonstrated moderate expression levels
- ***AnaD* stands out with the highest expression**, nearing 400 TPM, indicating steady involvement in toxin production.

Differences in metabolic pathways

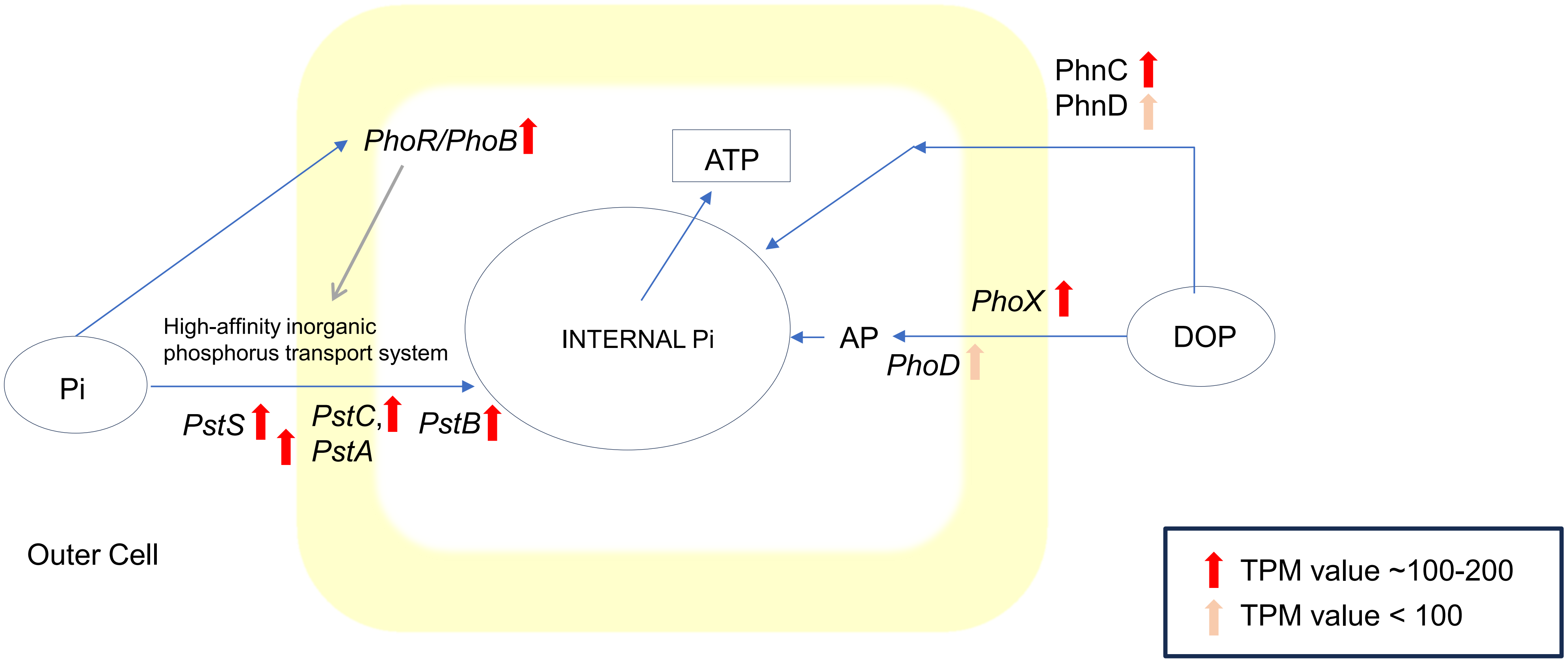
Microcoleus from our study has gained **additional thiamine biosynthesis genes** and **anti-drug resistance genes**, indicating potential adaptations for enhanced metabolic independence and resilience against environmental stressor



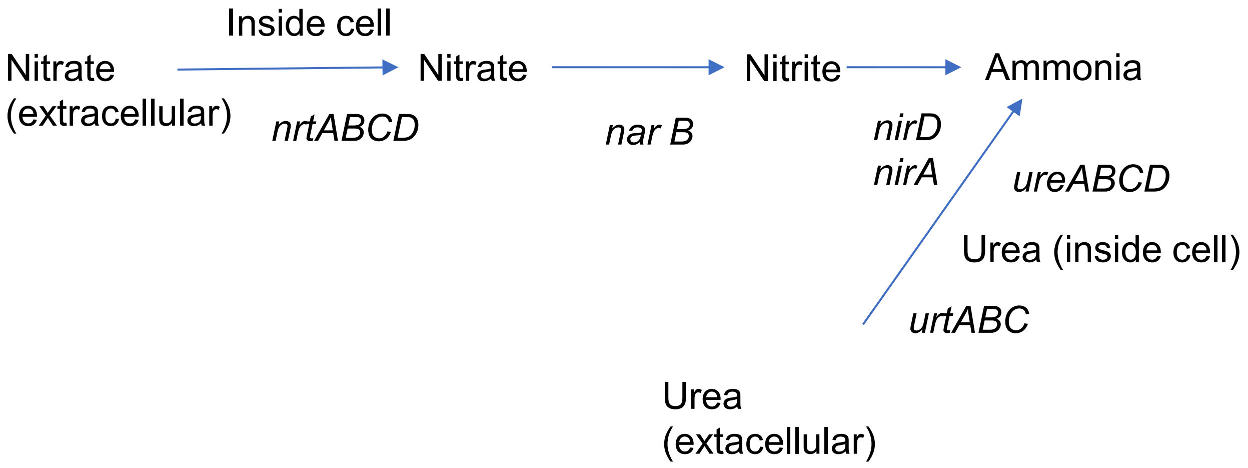
Microbe Annotator Results - Heatmap

Phosphorus acquisition in limiting P conditions

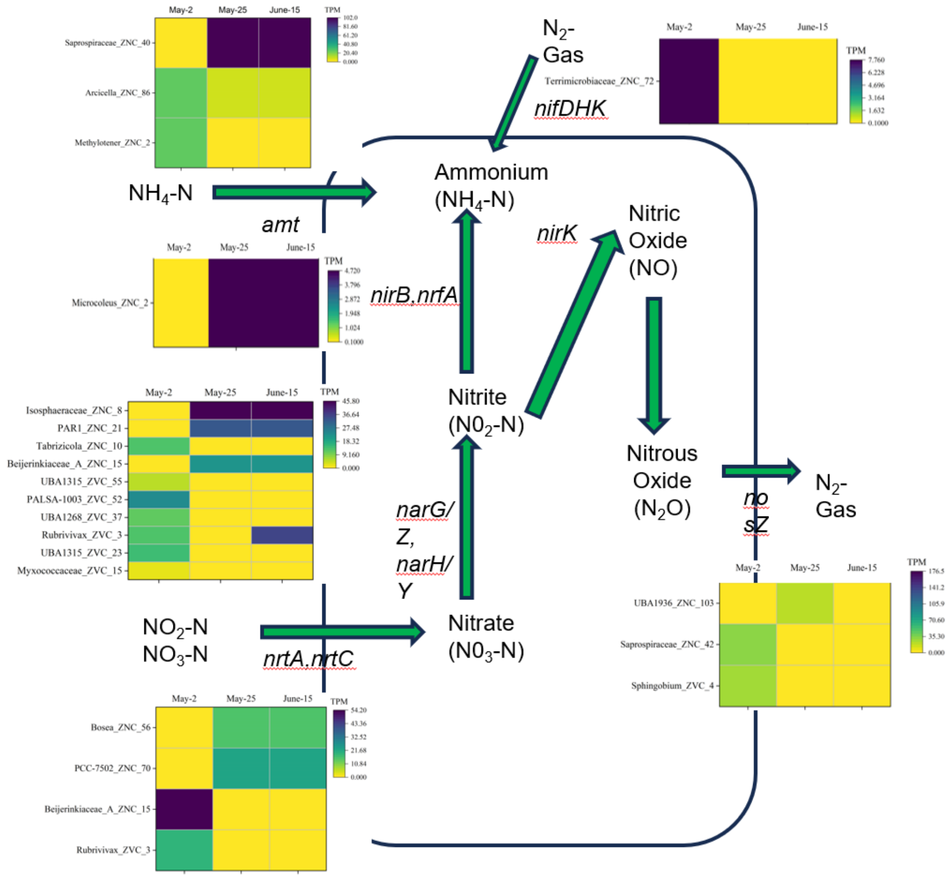
Microcoelus species thriving in Zion were found to **employ multiple pathways for phosphorus acquisition**.
The **high-affinity Pst system** exhibited moderate expression, alongside the activation of **alkaline phosphatase genes**
This allowed the organism to utilize both inorganic phosphate and organic phosphorus sources.



Nitrogen Acquisition in Benthic Mats



All the above genes were moderately expressed



The pathway and quantification of the transcripts of the genes involved in Nitrogen acquisition: A heatmap is used to graphically depict quantitatively evaluated transcripts. **The method for cyanobacteria and other bacterial species to get nitrogen is illustrated**. Rectangular symbols are used to clearly indicate these extracellular components of the process.

Summary of Findings

- A **single strain of *Microcoleus anatoxicus* was found** proliferating in a small side stream in Zion National Park.
- This strain produced **high levels of anatoxins within mats**, which **bled into the Virgin River**.
- **Thiamine biosynthesis genes** and **anti-drug resistance genes found.**
- **Active phosphorus acquisition** pathways were identified in the *Microcoleus* strain, showcasing its **adaptive mechanisms for nutrient uptake**.

Future direction

Better understanding of these nutrient cycling processes needed to improve ecosystem models and more accurately predict algal bloom events.

Questions?

References

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