Metabolic profiling to identify fundamental differences in toxic and non-toxic cyanobacterial strains

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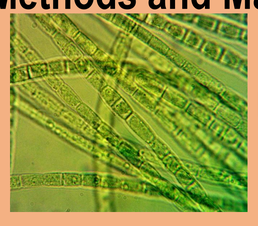
Introduction

- Of the pathogens that are commonly present in water, cyanobacteria is one that poses a significant health risk to the public and aquatic animals.
- Their potential existence in water is also a significant operational risk for water service providers to manage.
- Cyanobacteria produces toxins such as hepatotoxins, cytotoxin, neurotoxins and Dermatotoxin
- A big challenge for water industries is to provide safe drinking water during periods of cyanobacterial blooms when toxin levels are elevated in water (USEPA, 2018).
- Current methods for identifying and assessing pathogen viability and infectivity in water are time-consuming, expensive and can be unreliable.
- Omics is a broad technology which comprises genomics, transcriptomics, proteomics and metabolomics to detect genes, mRNA, proteins and metabolites, respectively, in a specific biological sample (Richard and Louise, 2011).
- Development of environmental metabolomics for the determination of different toxins, utilising the link between infectivity and increased metabolic activity is required.

Methods and Materials



Cyanobacteria

Anabaena cylindrica

Cylindrospermopsis raciborskii

Microcystis aeruginosa

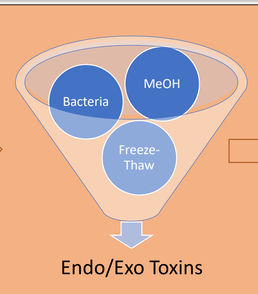
Nodularin spumigena

Growth Parameters

Temperature: 20°C, 28°C

Days: 7, 14

Light: 600 Lumens (12hr day/night cycle)

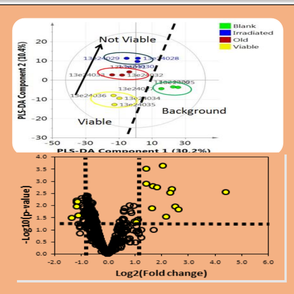


Endo/Exo Toxins

Toxin Extraction

(Christoffersen,Kaas, 2000).

Derivatization and GCMS processing

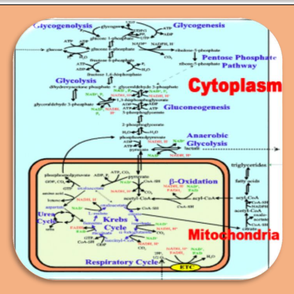


Uni and multi- variate statistics

PCA

PLS-DA

Volcano plot



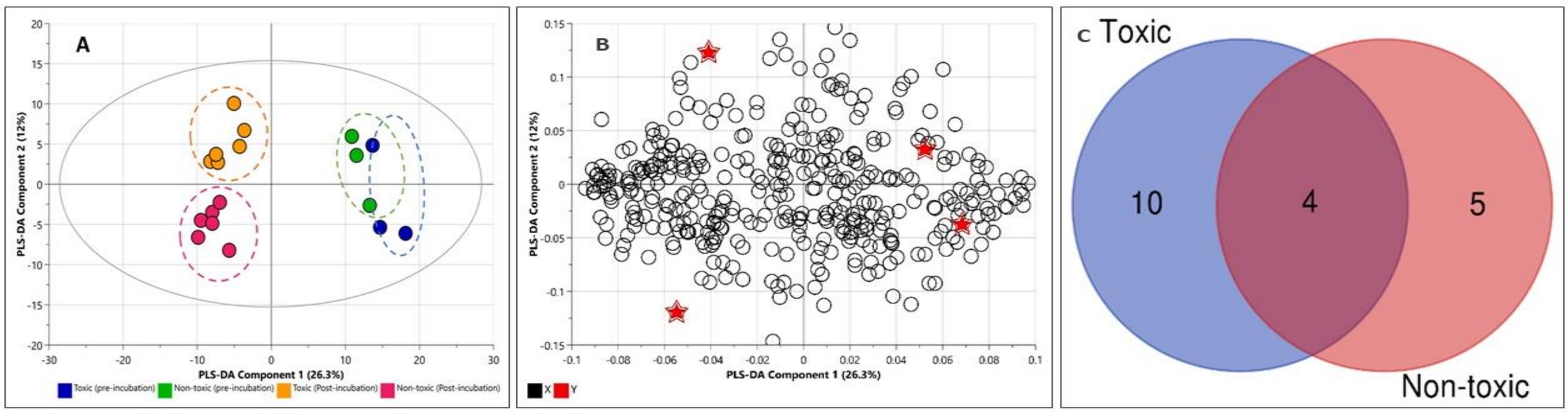
Characterising molecular cyanobacterial system.

Identify toxin producing parameters/pathways.

Identification of signature biomarkers.

Development of practical workflow and protocol for water Industries.

Findings



Multivariate analysis of metabolomic-derived datasets from cyanobacterial species at pre incubation (Day 0 at 28°C) and post-incubation (Day 7 and 14 at 28°C) ; **A**. Partial least square discriminant analysis (PLS-DA) scatter plot **B.** PLS-DA loadings plot. The white circles represent metabolites and the red stars represent cyanobacterial clusters. For the PLS-DA plots, the measures of fit were R2X[cum] = 0.49, R2X[cum] = 0.74 and Q2(cum) = 0.57 . The plot ellipse represents the 95% confidence interval as represented by Hotelling's T2 tolerance ellipse. **C.**Venn diagram showing unique and common significant metabolites in toxic and non-toxic strains of cyanobacteria.

Table 1: List of unique and common significant metabolites observed in toxic and non-toxic cyanobacterial strains

Unique Significant metabolites		
Toxic strains	**Non-toxic strains**	**Common**
D-Sedoheptulose-7-phosphate	Mevalonic acid	5-Tetrahydrocortisol
Adenosine	Octadecanedioic acid	C17 Sphingosine-1-phosphate
Uracil	Xylitol	5-beta Cholanic Acid
D-Glucose	Taurocholic acid	Linderatin
10-chloro-hexadecanoic acid	Vanillic acid	
3-phosphoserine		
3-deoxy-3-thiavitamin		
Egregiachloride B		
Hydroxytridecanoic acid		
9Z-Hexacosene		

Conclusions

- Comparison of toxic and non-toxic cyanobacterial metabolites was ~~done~~ successfully performed by LCMS metabolomics approach.
- Clear discrimination of toxic and non-toxic species based on metabolite profile was observed (Figure A).
- Signature metabolites (potential biomarker) identified by Chemometrics approach. (Figure C and Table 1).
- Unique and common significant metabolites identified in toxic and non-toxic cyanobacterial strains can be potential biomarkers (Table 1).

References

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Acknowledgements

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