

Investigation Of A Novel Toxin Produced By A Limnothrix Cyanobacteria

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Abstract

Limnothrix is a common genera of cyanobacteria that has previously shown very limited evidence as a producer of toxic metabolites. Recently, a species of *Limnothrix* cyanobacteria has been shown to be the producer of an unusual cyanobacterial toxin (the toxin suppresses cellular ATP production and inhibits protein synthesis). The identity of the *Limnothrix* toxin has not been previously characterised. An investigation into the isolation and identification of the toxic molecule produced by the *Limnothrix* cyanobacteria was the focus of this work.

Limnothrix "AC0243" cyanobacteria were cultured and harvested to produce cyanobacterial extracts containing the *Limnothrix* toxin. A protein synthesis inhibition bioassay utilising rabbit reticulocyte lysate was validated in this work as a means of guiding fractionation of the *Limnothrix* toxin.

Some physical properties of the *Limnothrix* toxin were investigated; the properties were approximate molecular mass, approximate temperature stability, solubility and decomposition upon exposure to acidic or basic pH solutions.

The cyanobacterial extract containing *Limnothrix* toxin was concentrated utilising solid phase extraction methodology; the solid phase extraction was performed on a variety of stationary phases but only diol-capped and ZIC-HILIC phases were able to demonstrate concentration of the *Limnothrix* toxin. Translation of the solid phase extraction methods using the diol-capped and ZIC-HILIC phases to a high performance liquid chromatography method was attempted. The translation was ultimately unsuccessful, but the investigation provided further information on the *Limnothrix* toxin. *Limnothrix* toxin demonstrated a very high affinity for polar stationary phases (e.g. silica, diol-capped silica, cyano-capped silica) when the mobile phase was at acidic pH; the high affinity was investigated to determine whether it would impact on concentration and purification of *Limnothrix* toxin.

The effectiveness of chromatographic partitioning performed on the diol-

capped and ZIC-HILIC stationary phases was analysed using mass spectrometry. The analysis was performed by examination of the population of total ion current profiles from fractions produced by the chromatographic methods employed. Solid phase extraction methods showed some evidence of partitioning while high performance liquid chromatography methods showed minimal evidence of partitioning or recovery of the active component.

Ultimately the *Limnothrix* toxin proved difficult to purify or characterise, however it was able to be concentrated by means of diol-capped solid phase extraction. Some recommendations for risk assessment in relation to the *Limnothrix* toxin are provided based on the evidence gathered during this project.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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Signed.....

Date.....

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Glossary of Abbreviations

ACE	Acetylcholineesterase
ADDA acid	3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic
ADWG	Australian drinking water guidelines
Arg	Arginine
ATP	Adenosine triphosphate
AX	Anion exchange
BMAA	B-Methylamino-L-alanine
CX	Cation exchange
CYN	Cylindrospermopsin
DBP	Disinfection byproduct
D-MeAs	D-Methylaspartate
DNA	Deoxyribonucleic acid
doCYN	Deoxycylindrospermopsin
ELISA	Enzyme linked immunosorbent assay
epiCYN	7-epicylindrospermopsin
GAC	Granular activated carbon
GC	Gas chromatography
GSH	Glutathione
GTP	Guanidine triphosphate
GTX	Gonyautotoxin
HEPES	2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid
HILIC	Hydrophilic interaction liquid chromatography
HPLC	High performance liquid chromatography
IC ₅₀	Concentration of sample that induces 50% inhibition
IR	Infrared
LD ₅₀	Dose lethal to 50% of test subjects
Lys	Lysine
NDMA	N-Methyl D-aspartate
NMR	Nuclear magnetic resonance
MIB	2-Methylisoborneol
NOM	Natural organic matter
MS	Mass spectrometry
mRNA	Messenger ribonucleic acid
PAC	Powdered activated carbon
PCR	Polymerase chain reaction
PDA	Photodiode array
PEG	Polyethylene glycol
PSA	Protein synthesis inhibition bioassay
PSP	Paralytic shellfish poison
SPE	Solid Phase Extraction
STX	Saxitoxin
tRNA	Transfer RNA
UV/VIS	Ultraviolet-visible