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ANTAGONISM OF *Bacillus* spp. TOWARDS *Microcystis aeruginosa*

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DECLARATION

I declare that the thesis, which I hereby submit for the degree *Philosophiae Doctor* at the University of Pretoria, Pretoria has not been previously submitted by me for a degree at another university.

_____ _____

J. R. Gumbo Date

SUMMARY

Freshwater resources are threatened by the presence and increase of harmful algal blooms (HABs) all over the world. The HABs are sometimes a direct result of anthropogenic pollution entering water bodies, such as partially treated nutrient-rich effluents and the leaching of fertilisers and animal wastes. *Microcystis* species are the dominant cyanobacteria (algae) that proliferate in these eutrophic waters. The impact of HABs on aquatic ecosystems and water resources, as well as their human health implications are well documented. Countermeasures have been proposed and implemented to manage HABs with varying levels of success. These control measures include the use of flocculants, mechanical removal of hyperscums and chemical algicides. The use of flocculants such as Phoslock™ is effective in reducing the phosphates in a water body thus depriving nutrients that are available to cyanobacteria. The mechanical option entails the manual removal of hyperscums thus reducing the numbers of cyanobacteria cells that may be the inoculum of the next bloom. The major disadvantage of these two measures is cost. Copper algicides have been used successfully to control HABs in raw water supplies intended for potable purposes. The major disadvantages are copper toxicity and release of microcystins from lysed cyanobacteria cells. Algicides accumulate in the sediments at concentration that are toxic to other aquatic organisms and may also cause long-term damage to the lake ecology. In some studies, microcystins have been implicated in the deaths of patients undergoing haemodialysis. Therefore there is an increasing need to reduce the use of chemicals for environmental and safety reasons. Thus, the development of environmentally friendly; safe non-chemical control measures such as biological control is of great importance to the management of HABs. Some papers, describe bacteria, which were isolated from eutrophic waters, such as *Sphingomonas* species with abilities to degrade microcystins and *Saprospira albida* with abilities to degrade *Microcystis* cells. Further research is required to evaluate whether these bacteria are antagonistic towards cyanobacteria. Ideally, a combination of strategies should be introduced; that is, combine predatory bacteria that directly lyse the cyanobacteria with microcystin degrading bacteria that then 'mop up' the released microcystins.

The major objective of this study was to isolate organisms that have a similar antagonistic properties; determine their mechanism of action and then develop a model to account for the interaction between the predator and prey as the basis for the development of a biological control agent.

During the screening for lytic organisms from eutrophic waters of Hartbeespoort dam, seven bacterial isolates were obtained. Based on electron microscope observation, two of the isolates were found aggregated around unhealthy *Microcystis* cells. These were identified as *Pseudomonas stutzeri* strain designated B2 and *Bacillus mycoides* strain designated B16. Based

on efficiency and efficacy experiments *B. mycoides* B16 was a more effective antagonist than *P. stutzeri* B2. Furthermore the *B. mycoides* B16: *Microcystis* critical ratio was found to be 1:1 in 12 days. Thus altering the predator-prey ratio by increasing the predator bacteria numbers reduced the *Microcystis* lysis time to six days. The *B. mycoides* B16 managed to reduce the population of alive *Microcystis* cells by 85% under turbulent conditions and 97% under static conditions in six days. The increase in predator bacteria numbers coincided with a decrease in growth of *Microcystis*.

The study on the interactions of *Microcystis aeruginosa* and *Bacillus mycoides* B16 indicated a series of morphological and ultrastructural changes within the cyanobacteria cell leading to its death. These are summarised in a conceptual model that was developed. The predatory bacteria, *B. mycoides* B16 attached onto the *Microcystis* cell through the use of fimbriae and or exopolymers. During this attachment the bacteria released extracellular substances that dissolved the *Microcystis* cell membrane and interfered with the photosynthesis process. The presence of numerous bacterial cells that aggregated around *Microcystis* cell provided a 'shade' that reduced the amount of light (hv) that reached the *Microcystis* cell. In response to these adverse conditions, the *Microcystis* cell did the following: It expanded its thylakoid system, the light harvesting system, to capture as much light as possible to enable it to carry out photosynthesis and it accumulated storage granules such as phosphate bodies, glycogen and cyanophycin and swollen cells. Other researchers have also reported the swelling phenomenon prior to cell lysis but did not account for what might be the cause. During the course of the lysis process the *Microcystis* cell underwent a transition stage that involved changes from alive (with an intact membrane) to membrane compromised (selective permeability), to death (no membrane) and eventual cell debris. Due to the depleted *Microcystis* cells, the *B. mycoides* B16 (non-motile, non-spore former) formed chains, i.e., exhibited rhizoidal growth in search of new *Microcystis* cells to attack.

In conclusion, the present evidence in this study suggests that *B. mycoides* B16 is an ectoparasite (close contact is essential) in its lysis of *Microcystis aeruginosa* under laboratory conditions. These findings that *B. mycoides* B16 is a predatory bacterium towards *Microcystis aeruginosa* need to be further evaluated under field conditions in mesocosm experiments (secluded areas in a lake) to determine the possibility of using this organism as a biological control agent.

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LIST OF ABBREVIATIONS

ABSA	American Biological Safety Association
BCECF-AM	2',7',-bis(2-carboxyethyl)-5-(and-6)-carboxyfluorescein acetoxymethyl ester
BCECF	2',7',-bis(2-carboxyethyl)-5-(and-6)-carboxyfluorescein
Calcein-AM	Acetoxymethyl ester
CDC	Centre for Diseases Control
CFDA	Carboxyfluorescein diacetate
CFDA-AM	Carboxyfluorescein diacetate acetoxy methyl ester
CTC	5-cyano-2,3,-ditolyl tretazolium chloride
CSE	Chemunex, Maisons-Alfort, France
CYN	cylindrospermopsin
DiOC6	3,3′-dihexyloxacarbocyanine iodide
DiBAC4	bis-(1,3-dibutylbarbituric acid) trimethine oxonol
DEAT	Departments of Environmental Affairs and Tourism
DWAF	Department of Water Affairs and Forestry
DWAF, RQS	Department of Water Affairs and Forestry, Resource Quality Services
DWA	Department of Water Affairs
EA	ENVIRONMENTAL AUTHORISATION
EEC	European Economic Community
FDA	fluorescence diacetate
FITC	fluorescein isothiocyanate
FISH	fluorescent *in-situ* hybridisation
FSC	forward scatter
Geosmin	trans-1, 10-dimethyl-trans-9-decalol
GMOA	Genetically Modified Organisms Act (Act 15 of 1997)
HAB	Harmful algal blooms
HRE	Host range expansion
HS	Host switching
HWAG	Hartbeespoort Water Action Group
LPS	Lipopolysaccharides
Microcystins-LR	Microcystins- (L for leucine and R for arginine)
MC	microcystins
2-MIB	2-methyl isoborneol
MRC	South Africa Medical Research Council

NDA	NATIONAL DEPARTMENT OF AGRICULTURE
NH_4	ammonium
NO_x	nitrates/nitrites
NEMA	National Environmental Management Act (Act 107 of 1998)
NEMBA	National Environmental Management: Biodiversity Act (Act 10 of 2004)
NEMP	National Eutrophication Monitoring Program
NWA	National Water Act (Act 36 of 1998)
NIWR	National Institute of Water Research
NIH	National Institute of Health
NHMRZ/ ARMCANZ	National Health and Medical Research Council, Agriculture and Resource Management Council of Australia and New Zealand
PSP	Paralytic shellfish poisons
PO_4P	phosphates
P	Phosphate levels
PAR	photosynthetically available irradiance
PI	propidium iodide
PMT	photomultiplier tube
PS II	photosystem II
Reglone A	diquat, 1,1-ethylene-2, 2-dipyridilium dibromide
Rh123	rhodamine 123
SEM	scanning electron microscopy
Simazine	2-chloro-4,6-bis(ethylamino)-s-triazine
SRP	soluble reactive phosphorus
TEM	transmission electron microscopy
TP	Total phosphorus
TSA	Tryptone Soy Agar
TSB	Tryptone Soy Broth
WTP 1	WATER TREATMENT PLANT NUMBER 1
WTP 2	Water treatment plant number 2
WHO	World Health Organization

PUBLICATIONS AND PRESENTATIONS

Published articles

1. Gumbo JR, Cloete TE, and Hall AN, (2006). Elucidation of the mechanism of cyanobacteria lysis of *Microcystis* after exposure to *Bacillus mycoides.* Proceedings of the *Microscopy Society of Southern Africa.* 36: 38.

2. Gumbo JR, Cloete TE, and Hall AN, (2004). The Algicidal effect of predatory bacteria on *Microcystis aeruginosa*. Proceedings of the *Microscopy Society of Southern Africa. 34: 34.*

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Articles submitted for publications

1. Gumbo JR, Ross G, and Cloete, TE, (xxxx). Biological Control of *Microcystis* dominated harmful Algal Blooms. **Submitted** to the Journal of Harmful Algae.

2. Gumbo JR, Ross G, and Cloete, TE, (xxxx). The isolation and identification of predatory bacteria from a *Microcystis* algal bloom. **Submitted** to the Journal of Water SA.

Articles in preparation

1. Gumbo JR, and Cloete, TE, (xxxx) Chapter 4: Electron Microscope Assessment of the lytic activity of bacteria on *Microcystis.* In preparation.

2. Gumbo JR, Cloete, TE, Van Zyl GJJ, Sommerville J, (xxxx) Chapter 5: Flow cytometry measurements on Microcystis cells after exposure to predatory bacteria. In preparation.

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1. Gumbo JR, and Cloete TE, (2006). A flow cytometric technique to assess viable and membrane compromised cells of *Microcystis aeruginosa* upon predation by a biological control agent: *Bacillus mycoides*. (Oral presentation). International Conference and Exhibition on Water in the Environment. 20-22 February. Stellenbosch, South Africa.

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www.iwaconferences.co.za/abstracts/waterp/abstract%20Emslie%20Gumbo%20Cloete.doc

Awards

Second best student poster at The 12th International Conference on Harmful Algae. 4-8 September, 2006. Copenhagen, Denmark.