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INHIBITORY METABOLITES PRODUCTION BY THE
CYANOBACTERIUM *Fischerella muscicola*

A thesis presented in partial fulfilment of
the requirements for the degree of
Doctor of Philosophy in Biotechnology
and Bioprocess Engineering at Massey University.

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Dedication

This study is dedicated to my Guru
Pandit Sri Ram Sharma Acharya and Vandaniya Mataji.

Without their blessings this study would have been impossible.

ABSTRACT

Studies have been carried out on the production and toxicity of inhibitory metabolites by cyanobacteria.

In the present study a detailed screening system was used to detect antimicrobial substances produced by the cyanobacteria. A range of cyanobacterial species were screened for the production of inhibitory metabolites. However, a majority of the cyanobacterial species screened showed no evidence of production of antimicrobial substances under the culture conditions used. However, *Fischerella muscicola* (UTEX 1829) produced antimicrobial metabolites and this cyanobacterium became the subject of further studies.

Preliminary characterization of both, intracellular and extracellular crude methanolic preparation from the cultures of cyanobacterium *Fischerella muscicola* (UTEX 1829) showed the inhibition of a range of cyanobacteria, eukaryotic algae and eubacteria, using an agar zone inhibition technique.

Using HPLC and GCMS techniques it was possible to demonstrate unique characteristics of these inhibitory metabolites in the crude methanolic cell extracts prepared from cultures of *F. muscicola*. The application of the GCMS technique confirmed the absence of fischerellin from the cultures of *F. muscicola* grown under various laboratory conditions used in these studies.

The effect of culture age on the production and leakage of inhibitory (toxic) metabolites was investigated in batch cultures. The total toxicity of *F. muscicola* was found to reach its maximum during the late exponential or early stationary phase of the growth. The higher toxicity was always found to be associated with the cell extracts rather than the cell-free culture broths indicating that a lower amount of leakage of the toxic material into the culture medium had occurred. However, there was a higher amount of toxicity found in the cell free culture broth extract when the culture was in a state of senescence.

Factorial experiments with the split plot design for the study of effects of various environmental factors such as light intensity, temperature, nitrogen and phosphorus on the production of inhibitory (toxic) metabolites, revealed that low light intensity ($10 \mu\text{mol m}^{-2} \text{s}^{-1}$) together with low temperature (10°C) significantly enhanced the toxicity of *F. muscicola*. Variations in the concentrations of the major nutrients, nitrogen and phosphorus did not have any significant effect on the toxicity of the cyanobacterium over the concentration ranges investigated.

The effect of various environmental factors on the release of inhibitory (toxic) metabolites from the cells of *F. muscicola* was investigated and it was found that the release of metabolites was enhanced under high light intensity ($60 \mu\text{mol m}^{-2} \text{s}^{-1}$) and higher temperature (30°C). However, at low light intensity ($10 \mu\text{mol m}^{-2} \text{s}^{-1}$) and low temperature (10°C) the release was minimal.

Under sets of conditions which generated the highest observed toxicity and the lowest observed toxicity in this cyanobacterium, it was found that growth of the organism was limited by the temperature.

This research contributes to an increased in the understanding of the physiology of *F. muscicola* in particular and to the toxic cyanobacteria in general. Furthermore, this new knowledge will contribute to a deeper understanding of the ecology of these important microorganisms.

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Table of Contents

Dedication	I
Abstract	II
Acknowledgements	V
Table of Contents	VI
List of Tables	XV
List of Figures	XVIII
Chapter 1 Introduction	1
Chapter 2 Literature Review	5
2.1 CYANOBACTERIA	6
2.1.1 Ecology	6
2.1.1.1 Factors influencing distribution	6
2.1.1.2 Light and its importance	8
2.1.1.3 Temperature and growth	8
2.1.1.4 pH	9
2.1.1.5 Nutrient preferences	9
2.1.2 The Toxic cyanobacteria	11

2.1.2.1 Activity and structure of cyanobacterial toxins	15
2.1.2.2 Cytotoxins	15
2.1.2.2.1 Cyanobacterin	16
2.1.2.2.2 Tolytoxin	18
2.1.2.2.3 Scytophycins	19
2.1.2.2.4 Acutiphycins	20
2.1.2.2.5 Hapalindole	21
2.1.2.3 Biotoxins	24
2.1.2.3.1 Neurotoxins	26
2.1.2.3.2 Hepatotoxins	30
2.1.2.3.2.1 Microcystins	30
2.1.2.3.2.2 Nodularins	32
2.1.2.4 Lipopolysaccharides (Endotoxins)	35
2.1.2.5 Marine cyanobacterial toxins	36
2.1.2.6 Regulation of toxin production	38
2.1.2.6.1 Field studies	39
2.1.2.6.2 Laboratory studies	41
2.1.2.7 Fate and biological significance of cyanobacterial toxins . .	43
2.1.2.7.1 Release of toxins into the water	43
2.1.2.7.2 Effect of toxins on aquatic life and food chains	44
2.1.2.7.3 The biological functions of toxins	45
2.1.2.8 Procedures for detection of cyanobacterial toxins	46
2.1.3 Aims of this investigation	49

Chapter 3	General Materials and Methods	52
3.1	ORGANISMS	53
3.1.1	Cyanobacteria	53
3.1.2	Characteristics of genus <i>Fischerella</i>	53
3.1.3	Chlorophyceae (Green algae)	56
3.1.4	Xanthophycean alga	56
3.1.5	Eubacteria	56
3.2	CULTURE MEDIA	57
3.2.1	For cyanobacteria (fresh water)	57
3.2.2	For cyanobacteria (marine)	59
3.2.3	For chlorophycean and xanthophycean algal cultures	62
3.2.4	For eubacterial cultures	62
3.3	CHEMICALS	63
3.4	STERILIZATION OF CULTURE MEDIA	63
3.5	CLEANING OF GLASSWARE	64
3.6	GROWTH CONDITIONS FOR CYANOBACTERIAL AND ALGAL CULTURE	64
3.7	INOCULUM PREPARATION OF CYANOBACTERIAL CULTURES FOR EXPERIMENTAL FLASKS	65
3.8	GROWTH CONDITIONS FOR BACTERIAL CULTURES	66
3.9	GROWTH MEASUREMENT PARAMETERS	66
3.9.1	Determination of Biomass Concentration	66
3.9.2	Optical density measurement of cyanobacterial growth	67
3.9.3	Cell counting for chlorophycean alga <i>Scenedesmus</i> sp.	67

	IX
3.9.4 Protein determination	67
3.10 OTHER ANALYTICAL METHODS	68
3.10.1 Measurement of Dissolved Oxygen	68
3.10.2 pH measurement	69
3.10.3 Microscopic examination	69
3.11 CYANOBACTERIAL TOXICITY TESTING	69
3.11.1 Preparation of cyanobacterial samples	70
3.11.2 Agar zone diffusion method	71
3.11.3 Toxicity testing of crude methanolic cell extracts using liquid culture	72
3.12 TOXICITY TESTING OF PURE HEPATO- AND NEURO- TOXIN OF CYANOBACTERIA AGAINST GREEN ALGAE	73
3.13 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) ANALYSIS OF METHANOLIC CRUDE EXTRACT OF <i>F. muscicola</i>	74
Chapter 4 Preliminary Screening for Toxin Producing Cyanobacteria . . .	75
4.1 INTRODUCTION	76
4.1.1 Liquid culture method	77
4.1.2 Agar zone diffusion method	77
4.2 MATERIAL AND METHODS	80
4.2.1 Pure toxins	80
4.2.2 Organisms and growth conditions	80

	X
4.2.3 Sample collection and preparation	80
4.2.4 Antimicrobial assays	81
4.2.5 Growth measurement parameters	82
4.2.5.1 Cell Counts	82
4.2.5.2 Optical density measurement	82
4.2.5.3 Calculation of specific growth rates	83
4.3 RESULTS	84
4.3.1 Effect of commercial toxins on the target organism	84
4.3.1.1 Effect of Anatoxin-a	84
4.3.1.2 Effect of Microcystin-lr and -rr on the growth of <i>Scenedesmus sp.</i>	87
4.3.2 The screening of NZ isolates of cyanobacteria for toxin production	91
4.3.2.1 Shake flask (liquid) culture detection of toxins	91
4.3.2.2 An agar diffusion zone assay system for toxin detection . .	97
4.3.2.3 Screening of UTEX cultures for toxin production	98
4.3.2.3.1 <i>Anabaena flos-aquae</i> (UTEX 1444)	99
4.3.2.3.2 <i>Fischerella muscicola</i> (UTEX 1829)	101
4.3.2.3.3 <i>Fischerella ambigua</i> (UTEX 1903)	102
4.4 DISCUSSION	106
 Chapter 5 An Analysis of Toxic Material from <i>F. muscicola</i>	110
5.1 INTRODUCTION	111
5.1 MATERIALS AND METHODS	113

5.1.1 Organisms and growth conditions 113

5.1.2 Antimicrobial activity determination 113

5.1.3 High Performance Liquid Chromatography (HPLC) of
methanolic crude cell extract of *F. muscicola* 113

5.1.3.1 Isocratic run 113

5.1.4 Gas Chromatography-Mass Spectrometric determination of
fischerellin 115

5.1.4.1 Experimental 1. 115

5.1.4.2 Experimental 2. 115

5.2 RESULTS 116

5.2.1 Results from high performance liquid chromatography
(HPLC) 116

5.2.2 Results from gas chromatography/mass spectrometry (GC-
MS) 120

5.3 DISCUSSION 129

Chapter 6 Growth and toxin production by *Fischerella muscicola* in batch

culture 131

6.1 INTRODUCTION 132

6.2 MATERIAL AND METHODS 135

6.2.1 Organisms and growth conditions 135

6.2.1.1 Culture source 135

6.2.1.2 Culture maintenance 135

6.2.1.3 Inoculum preparation 135

6.2.2 Measurement of growth parameters	135
6.2.3 pH measurement	136
6.2.4 Toxicity measurement	136
6.2.5 Presentation of Data	136
6.3 RESULTS	138
6.3.1 Preliminary determination of growth and toxicity of <i>F.</i> <i>muscicola</i>	138
6.3.2 Quantifying Growth of <i>F. muscicola</i> (Choice of Method) . . .	144
6.3.3 Growth and toxicity of <i>F.muscicola</i> in batch culture	147
6.3.3.1 Extractable Intracellular Toxicity	147
6.3.3.2 Specific Toxicity	148
6.3.3.3 Diffusible (Supernatant) Toxicity	150
6.3.3.4 Supernatant pH and Dissolved Oxygen Concentrations . . .	150
6.4 DISCUSSION	153
Chapter 7 Effect of Environmental Factors on Toxins Production.	156
7.1 INTRODUCTION	157
7.2 MATERIAL AND METHODS	162
7.2.1 Inoculum preparation	162
7.2.2 Sample collection and culture preparation	163
7.2.3 Toxicity measurement	163
7.2.4 Measurement of growth parameters	164
7.2.5 Experimental design	164
7.2.6 Analysis of a split plot experiment	169

7.3 RESULTS	170
7.3.1 Effect of light and Temperature	170
7.3.1.1 The main effect of temperature on the toxicity	172
7.3.1.2 The main effect of light on the toxicity	173
7.3.2 The main effect of nitrogen on the toxicity	175
7.3.3 The main effect of phosphorus on the toxicity	175
7.3.4 Effect of various physical and chemical factor interaction terms on the toxicity	175
7.3.4.1 The interaction effects of nitrogen and phosphorus on culture toxicity	176
7.3.4.2 Interaction effects of light and nitrogen on culture toxicity	176
7.3.4.3 The interaction effects of temperature and nitrogen on culture toxicity	178
7.3.4.4 The interaction effects of light, temperature and phosphorus	180
7.3.5 The leakage of <i>F. muscicola</i> toxins into the culture medium	187
7.3.6 Growth curve of <i>F. muscicola</i> under conditions leading to highest and lowest toxicity in the cyanobacterium	190
7.4 DISCUSSION	192
 Chapter 8 General Discussion	 199

Chapter 9 Conclusions 208

Chapter 10 Recommendations 211

Bibliography 214

Appendices 246

 Appendix A Standard curve of protein 247

 Appendix B SAS Programming 249

 Appendix C Pairwise comparison of LIT*TMP*PH 253

List of Tables

Table 2.1. Countries in which toxigenic cyanobacteria have been reported in Fresh or Marine Waters (adapted from Carmichael, 1989).	13
Table 2.2. Characteristics of water-soluble toxins from cyanobacteria (Ikawa & Sanser, 1990).	34
Table 3.1. Composition of Allen's medium (Star & Zeikus, 1987).	57
Table 3.2. PIV metal solution (Star & Zeikus, 1987).	58
Table 3.3. Composition of Bold's 3N Bristol's (Star & Zeikus, 1987).	59
Table 3.4. ES-Enrichment for sea water medium (Star & Zeikus, 1987).	60
Table 3.5. Composition of PII metal solution (Star & Zeikus, 1987).	61
Table 3.6. Composition of Brain Heart Infusion (BHI) medium.	62
Table 4.1. The specific growth rates of <i>Scenedesmus</i> sp. in the presence of Anatoxin-a.	85
Table 4.2. The specific growth rates of <i>Scenedesmus</i> sp. in the presence of microcystins.	90
Table 4.3. The specific growth rates of <i>Scenedesmus</i> sp. in the presence of cyanobacterial extracts.	96
Table 4.4. The specific growth rates of a <i>Scenedesmus</i> sp. growing in the presence of <i>Nodularia</i> extracts.	97
Table 4.5 Antimicrobial activity of cyanobacterial cell extracts or culture broth tested against green alga <i>Scenedesmus</i> sp. using an agar diffusion method.	98
Table 4.6. The specific growth rates of <i>Scenedesmus</i> sp. in the presence of extracted material from <i>Anabaena flos-aquae</i>	101

Table 4.7. Inhibition of growth of different indicator organisms by <i>F.muscicola</i>	104
Table 4.8. Inhibition of growth of different indicator organisms by <i>F.muscicola</i>	105
Table 5.1. Inhibition of growth of indicator strain <i>Anabaena flos-aquae</i> by HPLC fractions of methanolic crude extracts prepared from the culture of <i>F.</i> <i>musciola</i>	117
Table 6.1. Distribution of microcystin during laboratory culture of <i>Microcystis</i> <i>aeruginosa</i> (from Anon., 1990).	134
Table 6.2. Cytotoxicity of cell extracts of <i>F. musciola</i> grown in shake flask and tested against the indicator organism <i>A. flos-aquae</i>	139
Table 6.3. Ratio of Extractable Toxicity (inhibition zone diameter) versus biomass of <i>F. musciola</i>	148
Table 6.4. Cytotoxicity of extracts of <i>F. musciola</i> grown in shake flask and tested against the indicator organism <i>A. flos-aquae</i>	152
Table 7.1 Values of light and temperature used.	167
Table 7.2 Concentration of each nutrient factor at their low, original and high levels.	168
Table 7.3 Arrangement of all possible combinations of nitrogen and phosphorus.	169
Table 7.4. ANOVA Table showing the overall effect of various factors and their combinations on the toxicity of <i>F. musciola</i>	171
Table 7.5. Pair-wise comparison of LS Means of Light and Temperature combinations.	172

Table 7.6. LS Means of light, temperature and phosphorus combinations.	182
Table 7.7. LSMeans for LIT*TMP*NG*PH showing all possible treatment combinations and their mean toxicity values.	184
Table 7.8 Mean inhibition zone diameters (Toxicity) of methanol extracted cell free culture broths from <i>F. muscicola</i> grown under different environmental conditions.	188

List of Figures

Figure 2.1. Diagrammatic representation of a cyanobacterial cell. CM, Cell membrane; TH, thylakoid; PB1 & PB2, front and side views, respectively, of two rows of phycobilisomes; GG, glycogen granules; CY, cyanophycin granule; P polyphosphate granule; R, 70S ribosomes; C, carboxysome; G, gas vesicle. (adapted from Stanier and Cohen-Bazire, 1977)	7
Figure 2.2. Molecular structure of the halogenated metabolite "cyanobacterin" produced by the freshwater cyanobacterium <i>Scytonema hofmanni</i> (Gleason & Wood, 1987).	17
Figure 2.3. Molecular structure of tolytoxin produced by the cyanobacteria <i>Scytonema sp.</i> and <i>Tolypothrix sp.</i> (Patterson & Carmeli, 1992).	19
Figure 2.4 Molecular structure of scytophycin B.	20
Figure 2.5. The cytotoxins of <i>Oscillatoria acutissima</i> , namely acutiphycin and didehydroacutiphycin (Barchi <i>et al.</i> , 1984).	21
Figure 2.6. Molecular structures of hapalindole A and hapalindolinone A and B, the cytotoxins produced by the filamentous cyanobacteria <i>Hapalosiphon fontinalis</i> and <i>Fischerella</i> (ATCC 53558) (Moore <i>et al.</i> , 1984; Schwartz <i>et al.</i> , 1987).	23
Figure 2.7. Molecular structures for the three lipophilic toxins from <i>Fischerella ambigua</i> : ambigol A (1); ambigol B (2) and tjipanazole D (3). All molecules are bioactive (Falch <i>et al.</i> , 1995).	25
Figure 2.8. Molecular structure of Anatoxin-a produced by <i>Anabaena flos-aquae</i> (Carmichael, 1992).	27

Figure 2.9. Molecular structure for Anatoxin-a(s) of <i>Anabaena flos-aquae</i> and other species of <i>Anabaena</i> (Carmichael, 1994).	28
Figure 2.10. Molecular structure for Aphantoxins produced by <i>Aphanizomenon flos-aquae</i> (Carmichael, 1992, 1994).	29
Figure 2.11. Molecular structure for the most commonly occurring microcystin. This is one of a number of toxic peptides produced by species of <i>Microcystis</i> (Kotak <i>et al.</i> , 1995).	32
Figure 2.12. Three naturally occurring nodularins originally discovered in <i>Nodularia spumegina</i>	33
Figure 2.13. Structure of debromoaplysiatoxin, lyngbyatoxin A and teleocidin B, all produced by marine cyanobacterium <i>Lyngbya majuscula</i> (Moore, 1981).	37
Figure 2.14. General scheme of toxin pathways through the food chain (adapted from Ikawa and Sanser 1990)	50
Figure 3.1. Light micrograph of filamentous cyanobacterium <i>Fischerella muscicola</i> (UTEX 1829), grown in Allen's medium (5 days old culture) at light intensity of $10 \mu\text{mol m}^{-2}\text{s}^{-1}$ and temperature 10°C (400 x magnification).	55
Figure 4.1. The growth of the target green alga <i>Scenedesmus</i> sp. in the presence of varying concentrations of anatoxin-a. (a) Control flasks for each experimental flask, (b) test flasks which contained up to $20 \mu\text{g/ml}$ of anatoxin-a.	86
Figure 4.2. The growth of the target green alga <i>Scenedesmus</i> sp. in the presence of varying concentrations of the hepatotoxin microcystin-lr. (a) Control flasks, (b) test flasks which contained the microcystin-lr.	88
Figure 4.3 The growth of the target green alga <i>Scenedesmus</i> sp. in the presence of	

varying concentrations of the hepatoin microcystin-rr.	89
Figure 4.4 Growth of the indicator alga <i>Scenedesmus</i> sp. in Allen's medium containing material from cyanobacterium <i>Scytonema</i> prepared as methanol extracts. (a) No added cell extract, (b) cell extract present.	93
Figure 4.5 Growth of the indicator alga <i>Scenedesmus</i> sp. in the presence of methanolic crude extracts prepared from <i>Oscillatoria</i> species (a) M193rt and (b) M194rt.	94
Figure 4.6 Growth of <i>Scenedesmus</i> sp. in Allen's medium, Allen' medium with methanol (+) and Allen's medium containing methanol extract of <i>Nodularia spumigena</i>	95
Figure 4.7 The growth of <i>Scenedesmus</i> sp. (a) control flasks, (b) methanolic crude extracts prepared from <i>Anabaena flos-aquae</i>	100
Figure 5.1 HPLC chromatogram (C ₁₈ isocratic) of the methanolic extract from a culture of <i>F. muscicola</i> showing toxic fractions No. 2 & No. 3 at λ 267nm; mobile phase MeOH:H ₂ O, 99:1 (v/v); flow rate 1 ml/min; 100 μ l injection volume	118
Figure 5.2 HPLC chromatogram (C ₁₈ isocratic) of the methanolic extract from a culture <i>F. muscicola</i> showing the presence of absorbance at λ 415nm corresponding with toxic fraction No.2 & 3.HPLC condition as indicated in Fig 5.1.	118
Figure 5.3 HPLC chromatogram of fraction No. 5 (collected between 7 to 9 min) at λ 267 nm. HPLC conditions as indicated in Fig. 5.1 Injection volumes 10 μ l.	119
Figure 5.4 HPLC chromatogram of fraction No. 5 (collected between 7 to 9 min) at λ 267 nm and tested for absorbance at λ 415 nm. The peak at 7.89 min is absent. HPLC conditions as indicated in Fig. 5.1. Injection volume 10 μ l.	119
Figure 5.5 GC-MS chromatogram of toxic fraction No. 3.	122

Figure 5.6 Molecular ion scan of fraction No. 5, using DCI probe method for the detection of a signal at m/z 408.	123
Figure 5.7 GC-MS chromatogram of fraction No. 5	124
Figure 5.8 Molecular ion scan of toxic fraction No. 3, using DCI probe method for detection of a signal at m/z 408.	125
Figure 5.9 Molecular ion scan of toxic fraction No. 2, using DCI probe method for detection of a signal at m/z 408.	126
Figure 5.10 GC-MS chromatogram of toxic fraction No. 2.	127
Figure 5.11 Full scan of toxic fraction No. 2, using DCI probe method for the detection of a signal associated with m/z 408.	128
Figure 6.1 Toxicity of <i>F. muscicola</i> towards <i>Anabaena flos-aquae</i>	140
Figure 6.2 Growth curve of <i>F. muscicola</i>	140
Figure 6.3 Agar plates demonstrating the agar zone diffusion method for toxin production. Growth of <i>Anabaena flos-aquae</i> UTEX 1444 is drawn on Allen's Agar. (a) No cytotoxin in central well, (b), (c), (d), (e) cytotoxin in central well producing a qualifiable zone on inhibition.	141-143
Figure 6.4 Growth of <i>F. muscicola</i> as measured by (a) dry weight (mg/l).	145
Figure 6.4b Growth of <i>F. muscicola</i> followed by Protein measurements	146
Figure 6.5 Standard curve of Optical Density vs Dry weight. Correlation Coefficient (r) =0.94	146
Figure 6.6a Growth of <i>F.muscicola</i> as measured by OD changes at wavelength 750nm	149

Figure 6.6b Toxicity of *F. muscicola* culture towards *A. flos-aquae*. 149

Figure 6.7 pH and Oxygen curve of *F. muscicola* culture. 151

Figure 7.1 Diagrammatic representation of experimental runs showing the main
plot factors Light (L) and Temperature (T). 166

Figure 7.2. Toxicity variation at different levels of light and temperature. 174

Figure 7.3 Effect of nitrogen on the toxicity at two levels of light. 177

Figure 7.4. Effect of nitrogen on the toxicity at two levels of temperature. 179

Figure 7.5 Light intensity variation at low temperature (10°C). 183

Figure 7.6 Light intensity variation at high temperature (30°C) 183

Figure 7.7. Toxicity (Inhibition zone diameters) results of *F. muscicola* culture
broths as a response to different environmental growth conditions. 189

Figure 7.8. Growth curve of *Fischerella muscicola* under different environmental
conditions. 191