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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.

VISHAL C. SRIVASTAVA

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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 μmol m^{-2} 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 μmol m^{-2} s^{-1}) and higher temperature (30°C). However, at low light intensity (10 μmol m^{-2} 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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