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Comparative effects of the blue green algae *Nodularia spumigena* and a lysed extract on detoxification and antioxidant enzymes in the green lipped mussel (*Perna viridis*)

Warren R. Davies [a,*], William H.L. Siu [b], Ralph W. Jack [c], Rudolf S.S. Wu [b], Paul K.S. Lam [b,*], Dayanthi Nugegoda [a]

[a] *Department of Biotechnology and Environmental Biology, RMIT University, Melbourne, Victoria, Australia*
[b] *Department of Biology and Chemistry, City University of Hong Kong, Kowloon, Hong Kong*
[c] *Department of Microbiology, University of Otago, Dunedin, New Zealand*

Abstract

Nodularia spumigena periodically proliferates to cause toxic algal blooms with some aquatic animals enduring and consuming high densities of the blue green algae or toxic lysis. *N. spumigena* contains toxic compounds such as nodularin and lipopolysaccharides. This current work investigates physiological effects of exposure from bloom conditions of *N. spumigena* cells and a post-bloom lysis. Biochemical and antioxidative biomarkers were comparatively studied over an acute 3-day exposure. In general, a post-bloom *N. spumigena* lysis caused opposite physiological responses to bloom densities of *N. spumigena*. Specifically, increases in glutathione (GSH) and glutathione peroxidase (GPx) and decreases in glutathione *S*-transferase (GST) were observed from the *N. spumigena* lysis. In contrast, *N. spumigena* cell densities decreased GSH and increased GST and lipid peroxidation (LPO) in mussels. Findings also suggest that at different stages of a toxic bloom, exposure may result in toxic stress to specific organs in the mussel.

Keywords: *Nodularia spumigena*; Detoxification; Antioxidative enzyme; Nodularin

1. Introduction

Proliferation of Cyanobacteria, will occur under favorable conditions and are commonly known as algal blooms. Many cyanobacterial blooms are also well known producers of toxins. Investigations have shown that algal toxins have been the cause of poisonings and mortalities in animals (Kuiper-Goodman et al., 1999; Bell and Codd, 1994; Carmichael, 1992, 1989; Falconer et al., 1983) and illness or death in humans (Kuiper-Goodman et al., 1999; Pouria et al., 1998).

The cyanotoxin nodularin is produced from the genera *Nodularia* and is known to be hepatotoxic. Nodularin is a cyclic peptide, structurally similar to microcystin and has similar toxic action to okadiac acid (Gehringer, 2004). Toxicity is caused by specific inhibition of the serine-threonine protein phosphatases (PPs) leading to hyperphosphorylation of PPs and cell deformation and breakdown (Kaya, 1996).

Blooms of *N. spumigena* occur worldwide and some important aquatic species are affected by these toxic outbreaks. Rapid expansion of biomass sees many sessile animals ingest the cyanobacteria or exposed to the toxic lysis upon bloom degradation. Bioaccumulation of nodularin has been reported in fish e.g. snapper, tailor, garfish, flounder, Atlantic cod and threespine stickleback (Engström-Öst et al., 2002; Sipiä et al., 2002, 2001a,b;

* Corresponding authors. Tel.: +61 3 9925 7150; fax: +61 3 9925 7110 (W.R. Davies).
E-mail address: warren.davies@rmit.edu.au (W.R. Davies).

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Van Buynder et al., 2001), in crustaceans e.g. prawns (Van Buynder et al., 2001) and mysid shrimp (Engström-Öst et al., 2002) and molluscs such as mussel (Sipiä et al., 2002, 2001a,b; Van Buynder et al., 2001) and clam (Lehtonen et al., 2003; Sipiä et al., 2002). Many of these investigated species are an important food source for humans or aquatic species and vectorial transport of algal toxins through food webs has been of great interest to many researchers (Engström-Öst et al., 2002; Tester et al., 2000; Turner and Tester, 1997).

Although accumulation of nodularin in aquatic species appears well researched, less is known of the mechanisms to detoxify algal toxins and maintain a healthy status. In a bid to lower toxicity, cells have defense systems that modify toxic substances to aid in their excretion. Phase II activation of glutathione *S*-transferase and subsequent conjugation of glutathione to a xenobiotic increases the water solubility and ultimate excretion. Phase II detoxification and conjugates have been reported for cyanotoxins (Pflugmacher et al., 2001, 1999, 1998; Takenaka, 2001; Wiegand et al., 1999; Kondo et al., 1992), crude extracts (Best et al., 2002; Pietsch et al., 2001) and other cyanobacterial compounds (Wiegand et al., 2002).

During the course of detoxification, intermediates may also be formed that bring about cellular damage. Reactive oxygen species (ROS) can impart damage to a wide range of cellular components including DNA and is linked to many diseases in animals and humans including cancer (De Zwart et al., 1999). Ding et al. (1999) reported that *Microcystis* extracts had strong mutagenic and genotoxic effects and recently, antioxidant enzyme responses and measures of ROS formation have been reported for microcystin or *Microcystis* extracts (Botha et al., 2004; Bouaïcha and Maatouk, 2004; Chen et al., 2004; Wiegand et al., 2002; Pietsch et al., 2001; Ding et al., 1998) and for nodularin (Bouaïcha and Maatouk, 2004; Lankoff et al., 2002).

The aim of this study was to investigate *N. spumigena* toxicity at two different phases of a bloom. The effects of bloom conditions of *N. spumigena* and a post-bloom lysis of *N. spumigena* were comparatively investigated on a range of antioxidant and detoxifying enzymes in the Green lipped mussel (*P. viridis*). The mussel was chosen as the test model with in mind that this animal is typical of a sessile species that would endure blooms of *N. spumigena* and that reports of antioxidative and detoxification effects by *N. spumigena* are few in the mussel. The effects of *N. spumigena* were investigated in both the gill and hepatopancreas of the mussel. These two organs were selected as lysed constituents of *Nodularia* would be in close contact with the gills and that the hepatopancreas tends to actively accumulate nodularin, and is an important organ in digestion and detoxification. Treatments of *N. spumigena* used in this study were analogous to high bloom conditions (Van Buynder et al., 2001) and an extracted lysis of *N. spumigena* may simulate a post-bloom senescence and liberation of toxic components. Phase II detoxification in green lipped mussels were studied by measuring glutathione *S*-transferase (GST) and glutathione (GSH). Antioxidative responses were evaluated by measuring glutathione peroxidase (GPx) and lipid peroxidation (LPO).

2. Material and methods

2.1. Nodularia spumigena

Nodularia spumigena was collected using a 250 μm dip net during the February 2002 algal bloom in the Gippsland Lakes, Victoria, South-eastern Australia. Qualitative determination of *N. spumigena* was undertaken before the cyanobacterium was frozen at −80 °C. *N. spumigena* cell counts were measured in triplicate and the mean cell density calculated.

2.2. Preparation of N. spumigena lysed extract

Liberation of soluble contents from the cyanobacteria was similar to that of Pietsch et al. (2001), where cells were repeatedly freeze-thawed at −80 °C to room temperature, sonicated for 5 min in a Crest 950HT® ultrasonicator and homogenised for 2 min using a IKA T25® Ultra Turrex® homogeniser. The soluble fraction was centrifuged at 19,000 g for 20 min at 4 °C in a Beckman-Coulter® Alegra® 21R centrifuge to clarify the extract. The nodularin-bloom crude extract was stored in the dark at −20 °C for future application.

2.3. Determination of nodularin in a N. spumigena lysed extract

Although not all constituents of the algal lysis could be characterised, the main known toxic component (nodularin) was measured using a HPLC system, consisting of a Waters 600S controller, 626 pump, 717 autosampler and a Grom® 60 × 2 mm reverse phase column, packed with 3 μm Grom®-Sil 100 ODS. Nodularin detection employed a Waters 996 DAD multi-scanning UV detector, scanning a wavelength between 210 and 280 nm, with optimal wavelength detection set at 238 nm. Elution of analytes used a mobile phase gradient from 20:80 methanol and water run for 12 min. Nodularin in the crude extract was identified using pure nodularin standards (Calbiochem® USA).

2.4. Green lipped mussels (Perna viridis)

Green lipped mussels (*Perna viridis*) were sorted into a size class between 7–10 cm shell length and removed of epibionts and bysus. Mussels were acclimated for one