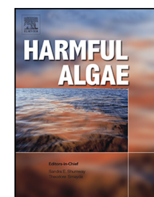
Harmful Algae 31 (2014) 177–187

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal



Suitability of a cytotoxicity assay for detection of potentially harmful compounds produced by freshwater bloom-forming algae

Ryan J. Sorichetti [a], Jace T. McLaughlin [a], Irena F. Creed [a,*], Charles G. Trick [a,b]

[a] Department of Biology, Western University, London, Ontario, Canada N6A 5B7
[b] Schulich School of Medicine and Dentistry, Western University, London, Ontario, Canada N6A 5B7

ARTICLE INFO

Article history:
Received 1 May 2013
Received in revised form 1 November 2013
Accepted 2 November 2013

Keywords:
Cyanobacteria
Freshwater
Harmful algal bloom
Rainbow trout Gill cytotoxicity assay
Taste and odor compounds
Toxins

ABSTRACT

Detecting harmful bioactive compounds produced by bloom-forming pelagic algae is important to assess potential risks to public health. We investigated the application of a cell-based bioassay: the rainbow trout gill-w1 cytotoxicity assay (RCA) that detects changes in cell metabolism. The RCA was used to evaluate the cytotoxic effects of (1) six natural freshwater lake samples from cyanobacteria-rich lakes in central Ontario, Canada; (2) analytical standards of toxins and noxious compounds likely to be produced by the algal communities in these lakes; and (3) complex mixtures of compounds produced by cyanobacterial and chrysophyte cultures. RCA provided a measure of lake water toxicity that could not be reproduced using toxin or noxious compound standards. RCA was not sensitive to toxins and only sensitive to noxious compounds at concentrations higher than reported environmental averages ($EC_{50} \geq 10^3$ nM). Cultured algae produced bioactive compounds that had recognizable dose dependent and toxic effects as indicated by RCA. Toxicity of these bioactive compounds depended on taxa (cyanobacteria, not chrysophytes), growth stage (stationary phase more toxic than exponential phase), location (intracellular more toxic than extracellular) and iron status (cells in high-iron treatment more toxic than cells in low-iron treatment). The RCA provides a new avenue of exploration and potential for the detection of natural lake algal toxic and noxious compounds.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Algal blooms are marked by significant increases in the population of pelagic algae resulting in the esthetic, odorous and/or biochemical fouling of surface waters (Reynolds and Walsby, 1975). Harmful algal blooms (HABs) are a sub-category of these events with most being distinguished by the presence of particular taxa at high levels of biomass that have the ability to produce toxins, irritants and/or noxious secondary metabolites in addition to other harmful effects including fish gill clogging, shading light access at depths and creating anoxic waters (Carmichael, 1992, 2001; Watson, 2003). Freshwater HABs can have serious ecological, toxicological and physiological effects on aquatic and terrestrial biota and increased occurrence of these events in North American over the past three decades poses cause for concern (Skulberg et al., 1984; Falconer, 1999; Carmichael, 2001, 2008; Paerl et al., 2001; Winter et al., 2011; Huber et al., 2012).

Blooms of high biomass are commonly attributed to excess nutrients entering water bodies (Heisler et al., 2008; Paerl and Huisman, 2008). HABs are formed when these nutrients are preferentially assimilated by harmful algal species that accumulate in biomass (Reynolds and Walsby, 1975; Paerl, 1988). Despite the multiple factors that promote HAB formation, accelerated eutrophication of surface waters by human inputs is the leading cause of HABs in freshwater systems (Schindler, 1987; Smith, 2003). Loading of macronutrients such as nitrogen (N) and phosphorus (P) from atmospheric and terrestrial sources is of particular concern and has been implicated as key nutrients for bloom development (Schindler, 1977; Guildford and Hecky, 2000).

Additional work has illustrated the importance of micronutrients in algal growth (Klausmeier et al., 2004; Molot et al., 2010; Fujii et al., 2011; c.f. Sorichetti et al., in press). Iron (Fe) has been identified as a critical micronutrient for bloom development and maintenance because it is required for essential cellular processes such as photosynthesis, pigment biosynthesis and (in some cyanobacteria) nitrogen fixation (Guikema and Sherman, 1983; Raven et al., 1999; Sterner et al., 2004). Fe has also been identified as a regulator of microcystin production, a potent cyanobacterial hepatotoxin (Kaplan et al., 2012). However, unlike most cellular functions, the production of microcystin increases when cyanobacteria are Fe-limited (Utkilen and Gjølme, 1995; Alexova et al.,

* Corresponding author at: Department of Biology, Western University, London, Ontario, Canada. Tel.: +1 519 661 4265.
E-mail addresses: rsorich@uwo.ca (R.J. Sorichetti), jmclau3@uwo.ca (J.T. McLaughlin), icreed@uwo.ca (I.F. Creed), cyano@uwo.ca (C.G. Trick).

1568-9883/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.hal.2013.11.001

2011; Kaplan et al., 2012). The role of Fe in chrysophyte noxious compound production is unknown.

Cyanobacteria are the most common group of algae associated with freshwater HABs. Common genera in lakes include: *Microcystis*, *Anabaena*, *Aphanizomenon* and *Gloeotrichia*; all of which have the potential to produce toxins, noxious and/or bioactive compounds (Carmichael, 2001; Paerl et al., 2001; Watson et al., 2008; Molot et al., 2010; Winter et al., 2011). The incidence of blooms dominated by chrysophyte algae may also be increasing in North America (Winter et al., 2011). Chrysophyte genera that are commonly observed dominating bloom events in lakes include: *Dinobryon, Synura, Uroglena* and *Mallomonas* (Nicholls, 1995; Watson, 2003; Paterson et al., 2004, 2008). While chrysophyte algae common to freshwaters do not produce toxins, they are infamous for their ability to produce a variety of noxious compounds that act as irritants or offensive taste and odor causing substances (Jüttner et al., 1986; Nicholls, 1995; Watson et al., 2008; Paterson et al., 2004).

This study addresses the need for quick, reliable and cost effective tools to detect and evaluate the cytotoxicity of freshwater samples that may be contaminated with toxic, noxious and/or bioactive algal metabolites. In 2008, Environment Canada released a report addressing the increase in HAB occurrence (Charlton et al., 2008). Three important research needs were outlined in this report, including: improving detection, characterization and modeling of toxic and noxious algal metabolites produced during HABs (Charlton et al., 2008). Recent research has investigated the ability of mammalian cell lines to detect the cytotoxic effects of compounds produced by marine algal isolates (Dorantes-Aranda et al., 2011) and this work has illustrated the potential for the adaptation of these assays to detect compounds produced by freshwater algae (Burkholder et al., 2005; Dorantes-Aranda et al., 2011). Adapting existing cell-based assays to quantify the toxicity of natural lake water samples is one way to improve detection and may offer a solution to the first step in the line of research needs. The low volume of sample required for analysis, the large number of samples that can be tested, the rapid exposure time, ease of sample preparation and avoiding the sacrifice of whole organisms are the main advantages of cell-based assays (Dayeh et al., 2005).

A cell-based assay of particular interest is the rainbow trout gill (RTgill-W1) cytotoxicity assay (RCA) that has been used in conjunction with various in vitro and in vivo methods of analysis to serve as a proxy and assess the effects of a wide range of environmental pollutants and toxicants on aquatic organisms such as fish and invertebrates (Lee et al., 2009). The RCA considers viability of cell metabolism using the reducing environment of cells to measure changes in cellular metabolic activity. Reduction in the rezasurin-containing fluorescent dye PrestoBlue™ is measured spectrofluorometrically as recovery from suspected cellular metabolic impairment can be evaluated by applying this dye to living cell lines. A decline in PrestoBlue™ reduction is thus indicative of cellular metabolic impairment. The adaptation of the RCA for investigations into polycyclic aromatic hydrocarbons, industrial effluents, petrochemicals, jellyfish venom and many other compounds occurring in both freshwater and marine systems has made it a prominent candidate for further use in freshwater research (Schirmer et al., 1998, 2001; Dayeh et al., 2005; Helmholz et al., 2010).

In this study, the effectiveness of the RCA is assessed by applying it to natural lake water samples, analytical standards of toxins and noxious compounds produced by freshwater algae and analysis of complex mixtures of metabolites produced by cultured freshwater algae, including both toxic and/or noxious compound-producing cyanobacteria and chrysophyte algae. To the best of our knowledge, this study represents the first application of the RCA using algal toxins and noxious compounds with related studies focusing primarily on the cellular effects of microcystins on fish liver cell lines (Boaru et al., 2006). A fish cell based bioassay is appropriate for this investigation because the toxic, noxious and bioactive compounds produced by cyanobacteria and chrysophytes are known to affect growth rate, modify behavior and exert histopathological effects in the liver, intestine, kidneys, heart, spleen and gills of fish and other aquatic organisms (Malbrouck and Kestemont, 2006).

The following hypotheses are tested:

H1. The RCA is effective in assessing cytotoxicity in natural lake water samples collected as a part of a routine water quality sampling strategy.

H2. The application of analytical standards of toxins and/or noxious compounds produced by freshwater algae will result in a concentration dependent decrease in the viability of cells in the RCA.

H3. The application of complex mixtures of algal metabolites extracted from cultures of toxic and/or noxious compound-producing cyanobacteria and chrysophyte algae will result in a concentration dependent decrease in viability as measured by the RCA where: (a) exposure solutions derived from exponential growth phases will result in significant reductions in cell viability relative to those of stationary growth phase; (b) exposure to solutions derived from lysed cultures (intracellular + extracellular compounds) will result in significant reductions in cell viability relative to solutions derived from non-lysed samples (extracellular compounds only); and (c) exposure solutions extracted from low Fe treatments (0.1 μM) will result in significant reductions in cell viability in relative to the high Fe treatments (10 μM).

Adapting the RCA to detect toxins, noxious and/or bioactive compounds produced by cyanobacteria and chrysophytes will provide insight into the additive, synergistic and/or antagonistic biological effects that these compounds may exhibit while produced in complex mixtures by various cyanobacteria and chrysophyte algae (Dayeh et al., 2005).

2. Materials and methods

2.1. Development of cytotoxicity assays

RTgill-W1 cells were obtained from the American Type Culture Collection (ATCC CRL-2523) (Bols et al., 1994). The cell line culture was maintained in the dark at 20 °C in sterile, plug sealed, tissue culture treated flasks containing a sterile hydrophilic surface that promotes cell attachment on the inside walls of the flask.

Cells were cryopreserved by suspending cell solutions in L-15 Complete-5% (v/v) dimethylsulfoxide (DMSO) medium at a density of 10^6 cells mL^{-1}. Aliquots (1 mL) were pipetted into 2 mL polypropylene Cryule® vials (985,746, Wheaton) and immediately sealed. A −1 °C minute^{-1} 'Mr. Frosty' freezing container (5100–0001, Thermo Fischer Scientific Inc.) filled with isopropyl alcohol was used to hold the vials and was immediately placed into −80 °C freezer. Cells remained viable in the freezer for a minimum of one year following initial freezing, as evidenced by successful culturing of cryopreserved cells. The absence of cell culture contamination by mycoplasma was confirmed through the use of a MycoAlert™ assay kit (LT07-118, Lonza) every two to three weeks. A 1 mL aliquot taken from the supernatant after the cell suspension was centrifuged at 200 × g for 5 min. The sample was combined with buffer solution and pipetted into a 96-micro well plate (MWP) in triplicate. Positive and negative control solutions were loaded into

Download English Version:

https://daneshyari.com/en/article/4545395

Download Persian Version:

https://daneshyari.com/article/4545395

Daneshyari.com