Harmful Algae 60 (2016) 157–166

Contents lists available at ScienceDirect

Harmful Algae

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

Characterization of total retinoid-like activity of compounds produced by three common phytoplankton species

Eliška Sychrová, Jana Priebojová, Marie Smutná, Kateřina Nováková, Jiří Kohoutek, Klára Hilscherová*

RECETOX – Research Centre for Toxic Compounds in the Environment, Masaryk University, Faculty of Science, Brno, Czechia

ARTICLE INFO

Article history:
Received 27 January 2016
Received in revised form 8 November 2016
Accepted 8 November 2016
Available online xxx

Keywords:
Retinoic acid receptor
Cyanobacteria
Biomass extracts
Exudates
All-*trans*-retinoic acid
9-*Cis* retinoic acid
Retinoid-like activity
Microcystin
Light conditions

ABSTRACT

Phytoplankton can produce various bioactive metabolites, which may affect other organisms in the aquatic environment. This study provides the first information on the total retinoid-like activity associated with both intracellular and extracellular metabolites produced by selected phytoplankton species that could play a role in teratogenic effects and developmental disruption in exposed organisms.

The studied species included a coccoid cyanobacteria (*Microcystis aeruginosa*), a filamentous cyanobacteria (*Aphanizomenon gracile*) and a green alga (*Desmodesmus quadricauda*), all of which commonly occur in freshwater bodies in Europe. Methanolic extracts from cellular material and extracellular exudates were prepared from cultures cultivated in two light-intensity variants with five replicates for each species. The retinoid-like activity was evaluated by *in vitro* assays along with chemical analyses of two potent retinoic acids (all-*trans* retinoic acid (ATRA) and 9cis-RA).

The mean total retinoid-like activity of metabolites produced by the three studied species representing different phytoplankton taxonomic groups ranged from 705 to 5572 ng ATRA equivalent/g dry matter corresponding to 0.064–0.234 ng ATRA/10^6 cells. Retinoid-like activity was found in the cellular extracts of all species, while only the extracellular exudates of cyanobacteria exhibited detectable activity (41–1081 ng ATRA/L). The greatest extracellular as well as total (extra- and intra- cellular together) retinoid-like activity was detected for *Microcystis aeruginosa*. The two potent retinoic acids studied were more frequently detected in cellular extracts than in extracellular exudates of all species. Their contribution to observed *in vitro* effects was relatively low for all tested samples (<10%), indicating a substantial contribution of other retinoid-like compounds to the overall activity. The results indicate possible influence of light intensity and cell density on the production of metabolites with retinoid-like activity and the cyanotoxin microcystin by the studied species.

The recalculation of the results per dry weight, water volume, per 10^6 cells and biovolume enables a direct comparison of the retinoid-like activity distribution between extracts and exudates and the use of the data for risk assessment in water bodies.

1. Introduction

Cyanobacterial blooms have become a global problem mainly due to the eutrophication of waterways (Heisler et al., 2008). Negative impacts of excessive cyanobacterial growth on ecosystems have been reported, such as a decrease in oxygen levels, changes in water chemistry or reduction of light penetration resulting in a decline of biodiversity. Furthermore, the production of biologically active metabolites can also affect surrounding organisms and the environment (Paerl et al., 2001; Smith, 2003). Cyanobacteria can produce bioactive compounds with various properties, *e.g.* cancer-promoting, mutagenic, anticancer, antibiotical, cytotoxic, genotoxic, hepatotoxic, neurotoxic, herbicidous, algicidous, virocidous, *etc.* (Prasanna et al., 2010). Each cyanobacterial isolate in a culture produces a set or pattern of metabolites that can define its so-called chemotype (Halstvedt et al., 2008).

Various cyanobacterial toxins can co-occur in the environment (Jančula et al., 2014). The most studied cyanotoxins are hepatotoxic and tumor-promoting microcystins. However, the effects of complex blooms often cannot be attributed solely to the activity of individual known cyanotoxins. In view of the fact that a lot of

* Corresponding author at: Research Centre for Toxic Compounds in the Environment, Faculty of Science, Masaryk University, Kamenice 753/5, pavillion A29, 625 00, Brno, Czechia.
E-mail address: hilscherova@recetox.muni.cz (K. Hilscherová).

http://dx.doi.org/10.1016/j.hal.2016.11.002
1568-9883/

studies have reported similar or even higher toxicity caused by unidentified cyanobacterial metabolites compared to the toxicity of detected previously identified cyanotoxins (*e.g.* microcystins-MCs, saxitoxin-SXT, cylindrospermopsin-CYN, anatoxin-ANA) (Falconer, 2007; Burýsková et al., 2006; Nováková et al., 2011), it is important to consider the production of further metabolites and their possible effects in the environment.

Disruptions in development and malformations in zebrafish *Danio rerio* embryos (*e.g.* tail, spine and mouth deformation, neural tube malformations, decelerated blood circulation) were observed after exposure to crude extracts or exudates of the cyanobacteria *Microcystis aeruginosa, Cylindrospermopsis raciborskii, Planktothrix agardhii, Aphanizomenon gracile* or *Aphanizomenon ovalisporum*, but the observed effects could not be explained by the known toxins (Acs et al., 2013; Berry et al., 2009; Jonas et al., 2015, 2014; Oberemm et al., 1997). Similarly in a study with frog *Xenopus laevis*, developmental deformities and other malformations were caused by cyanobacterial extracts independently of microcystins concentrations (Burýsková et al., 2006). Studies by Jonas et al. (2014, 2015) have reported endocrine disruptive activity caused by extracts or exudates of some cyanobacteria species. These studies showed the potency of cyanobacterial samples to cause specific effects at the organism cellular level (by activation of a retinoid receptor and estrogen receptor) as well as at the organism level (disruption of fish-embryo development). The malformations and developmental toxicity observed after exposure to cyanobacterial samples together with similar phenotypes caused by potent retinoid all-*trans* retinoic acid (ATRA) at comparable effective doses indicate that compounds with retinoid-like activity produced by some cyanobacterial species played a role in the observed effects (Jonas et al., 2015, 2014). Recently, the study of Millard et al. (2014) showed that some enzymes in the retinoic acid biosynthetic pathway in cyanobacteria are evolutionary close to their counterparts in eukaryotic organisms. Moreover, a few retinoic acid (RA) derivatives were identified by chemical analysis in cyanobacterial blooms from Taihu Lake in China, and in several laboratory cultures of cyanobacteria and algae (Wu et al., 2013, 2012). The retinoic acid analogue 7-hydroxy retinoic acid was newly identified in two species of cyanobacteria (Kaya et al., 2011).

Retinoids are important for many organisms; they play a crucial role in development as well as in maintenance of vital systems, such as the nervous, reproductive and immune systems. Their functions are mostly mediated through binding to nuclear transcription factors, retinoic acid receptors and retinoid X receptors, but they act also through some extranuclear mechanisms of action, *e.g.* modulation of protein kinase activity or covalent linking to regulatory proteins (reviewed by Blomhoff and Blomhoff, 2006). Retinoic acids (RAs) signaling is essential for normal vertebrate development (Stafford and Prince, 2002; Shimozono et al., 2013) and it is well conserved among different species (Rhinn and Dollé, 2012). RAs play an important role in the vertebrate development of the hindbrain, forebrain, fins and limbs and are responsible for the symmetry of the body axis (reviewed in Rhinn and Dollé, 2012). However, RAs represent strong teratogens when normal physiological concentrations are exceeded. Various deformities have been detected in embryos exposed to exogenous retinoids (Gardiner et al., 2003).

Retinoid-like activity associated with several species of cyanobacteria has been detected *in vitro* in a few previous studies. However these studies have focused separately on either the exudates (Jonas et al., 2014) or on biomass extracts (Kaya et al., 2011; Jonas et al., 2015) and used incomparable units expressed per volume or dry weight, respectively, which did not enable assessing the total production of compounds with retinoid-like activity or their distribution between biomass and exudates. Moreover, the few existing papers present data from one sample per species only, without any information on the variability. Regarding eukaryotic algae, there is no information on detection of retinoid-like activity, and the sole available previous study focused only on exudates which did not show detectable retinoid-like activity for any of the four studied algal species (Jonas et al., 2014). However, chemical analyses indicated the presence of a few retinoids, especially in the biomass of several algal species (Wu et al., 2013, 2012), which indicates the need to determine the total retinoid-like activity of algal biomasses. There is a lack of knowledge regarding the total retinoid-like activity associated with both intra- and extracellular metabolites together by phytoplankton species, its variability within the same species and across species, and also the factors influencing its intracellular and extracellular levels.

The quality and quantity of phytoplankton metabolites can be affected by external (abiotic and biotic) factors. The trends in production of various cyanobacterial compounds were shown to differ depending on the types of metabolites (*e.g.* cyanopeptolins, microviridins, MCs structural variants, pigments) (Bañares-España et al., 2013; Tonk et al., 2009, 2005; Wiedner et al., 2003), as well as on individual strains. Differences in toxin production were observed even between strains of the same cyanobacteria species, which responded differently to the same conditions (Pereira et al., 2012; Sivonen, 1990). Some studies concluded that the highest production of studied intracellular cyanobacterial metabolites (e.g MCs, ANA) takes place during the optimal growth period and that increases in the concentrations of extracellular cyanotoxins are time-dependent and probably related mainly to cell lysis (Kaebernick and Neilan, 2001; Rapala and Sivonen, 1997; Sivonen, 1996). Other studies found no clear correlation between growth rate and metabolite production and some studies have even indicated differing correlations for the same species (Agha et al., 2013; Griffiths and Saker, 2003; Preußel et al., 2009). Environmental conditions (temperature, nutrients, light) can influence the production of toxins (Griffiths and Saker, 2003; Horst et al., 2014). Among many studies, there is no uniformity of correlation between light conditions and toxin production (Bañares-España et al., 2013; Pereira et al., 2012; Tonk et al., 2009, 2005; Wiedner et al., 2003; Utkilen and Gjølme, 1992). A clearer impact on metabolite production was reported for the biotic factor cell density, which can influence the detected spectrum of metabolites as well as their quantity (Pereira and Giani, 2014). A higher content of peptides was documented in the laboratory cultivations with higher cell density of *M. panniformis*,*M. aeruginosa* and *Radiocystis fernandoii* (Pereira and Giani, 2014). Similarly, higher microcystin content was observed at higher cell density in field- and mesocosm-based studies (Wood et al., 2011, 2012).

Despite the important impact of retinoids on numerous physiological processes and development in exposed organisms, information related to the characterization of the retinoid-like activity of compounds produced by phytoplankton and the potential influence of any environmental conditions on its levels is rather limited.

The major aim of this study was to determine the total retinoid-like activity of metabolites (both intra- and extracellular together) produced by two cyanobacteria and one green alga chosen as common representatives of different taxonomic groups in surface water bodies. The levels were expressed per dry weight, water volume, but also per 10^6 cells and biovolume to enable direct comparison of the distribution of the retinoid-like activity between extracts of cellular material (further referred to as "extracts") and extracellular compounds produced out of cells (further referred to as "exudates") and the use of the data for the risk assessment in water bodies where the biomass density is generally monitored and reported as the number of cells per mL. The further goals were to (i) compare the retinoid-like potential in the extracts and exudates of the same cultivations, (ii) characterize