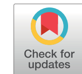
Algal Research 35 (2018) 508–518

Contents lists available at ScienceDirect

Algal Research

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

Allelochemicals from *Alexandrium minutum* induce rapid inhibition of metabolism and modify the membranes from *Chaetoceros muelleri*



Marc Long[a,b,*], Kévin Tallec[c], Philippe Soudant[a], Fabienne Le Grand[a], Anne Donval[a], Christophe Lambert[a], Géraldine Sarthou[a], Dianne F. Jolley[b], Hélène Hégaret[a,**]

[a] *Laboratoire des Sciences de l'Environnement Marin (LEMAR), UMR 6539 CNRS UBO IRD IFREMER – Institut Universitaire Européen de la Mer, Technopôle Brest-Iroise, Rue Dumont d'Urville, 29280 Plouzané, France*

[b] *School of Chemistry, University of Wollongong, NSW 2522, Australia*

[c] *Ifremer, Laboratoire des Sciences de l'Environnement Marin (LEMAR), UMR 6539 UBO/CNRS/IRD/Ifremer, 29280 Plouzané, France*

A R T I C L E I N F O

Keywords:
Allelopathy
Alexandrium
Metabolism
Photoinhibition
Lipids
Pigments

A B S T R A C T

Allelochemical interactions are likely to be a contributing factor explaining the success of large blooms of the harmful marine dinoflagellate *Alexandrium*, however, the physiological mechanisms of allelochemical interactions remain poorly described. Here we investigated the sub-lethal effects (on an hourly scale) of a filtrate containing allelochemicals from *Alexandrium minutum* on the physiology of the common diatom *Chaetoceros muelleri*. The filtrate induced deleterious effects to the diatom physiology within only 30 min of exposure. Esterase activity and photosynthesis were drastically inhibited, with up to 34% of the population being metabolically inactive and up to 30% reduction in photosystem II quantum yield when exposed to the filtrate. In addition, intracellular reactive oxygen species increased by 26% in response to allelochemical exposure. *C. muelleri* pigment and lipid analyses indicated that the photosystem II was inhibited, with photoinhibition-like responses (activation of xanthophyll cycles, and changes in associated lipids) upregulated to mitigate the toxic effects of allelochemicals. Changes in the proportions of membrane lipid classes and increased membrane fatty acids saturation by 9% may be an attempt to maintain membrane integrity and associated enzyme activity, or could be the result of deleterious effects on membranes. An 8% decrease in cellular storage lipids (triglycerides) revealed a mobilization of energy suggesting an energetic cost for the diatom to counteract the allelochemical effects. We hypothesize that the rapid alteration of physiological functions such as photosynthesis and some enzymatic activities may result from direct damage on external membranes. Overall this study describes the sub-lethal mechanisms and provides useful biomarkers to understand the role of allelochemical interactions and associated ecological processes in structuring plankton communities.

1. Introduction

The structure of plankton communities is shaped by complex interactions with their environment. The influence of abiotic factors, such as light, nutrients, seawater or stratification are well studied and known to have a strong role in community structure. The roles of biotic factors however are less well understood despite the increasing evidence that biotic factors are shaping plankton communities [1,2]. Chemical interactions between organisms (i.e. chemical ecology) are influencing the structure of marine communities. Chemical compounds can mediate communication between microbes, can play a role in sexual reproduction and competition between species or can be a chemical defense against grazers and pathogens [3–5]. Within this field, allelochemical interactions refer to the beneficial or adverse effects of chemicals (allelochemicals) released by a protist on the biology of co-occurring protists, predators or bacterial cells. The release of toxic allelochemicals gives the donor species a competitive advantage that could result in the formation of large blooms [6,7].

The genus *Alexandrium* is known to produce uncharacterized extracellular compounds with potent allelochemical activity. It is hypothesized that the allelochemical potency of this genus may enhance the establishment of large *Alexandrium* blooms [8]. The allelochemical potency is widespread among the genus *Alexandrium* as this activity was shown in at least 9 species: *A. minutum*, *A. fundyense*, *A. lusitanicum*, *A.*

* Correspondence to: M. Long, Laboratoire des Sciences de l'Environnement Marin (LEMAR), UMR 6539 CNRS UBO IRD IFREMER – Institut Universitaire Européen de la Mer, Technopôle Brest-Iroise, Rue Dumont d'Urville, 29280 Plouzané, France.

** Corresponding author.

E-mail addresses: marc.florian.long@gmail.com (M. Long), helene.hegaret@univ-brest.fr (H. Hégaret).

https://doi.org/10.1016/j.algal.2018.09.023
Received 21 March 2018; Received in revised form 31 July 2018; Accepted 26 September 2018
2211-9264/ © 2018 Elsevier B.V. All rights reserved.

affine, *A. ostenfeldii*, *A. pseudogonyaulax*, *A. tamarense*, *A. taylori* and *A. tamutum* [9–13]. Allelochemicals are released by cells into the surrounding environment and within minutes to hours induce cell lysis, cell immobilization and cyst formation [14]. Furthermore some of these allelochemicals may be responsible for other toxic effects on mammalian erythrocytes [15], and bivalve gametes [16]. They may also be responsible for reduced growth and behavior modification of the great scallop *Pecten maximus* [17] or deleterious effects on the physiology of the oyster *Crassostrea gigas* [18]. Nevertheless the nature and mode of action of those extracellular compounds remain unknown.

Researchers studying allelochemical interactions often quantify their effects on microalgal growth rate, membrane permeability and cell lysis in target cells however very few studies precisely elucidate the mode of action. For example, studies exploring allelochemical mechanisms from the genus *Alexandrium* have investigated their effects on photosynthesis [10], membranes [19] and transcriptomic [20], however the story is still incomplete. While the long-term (i.e. mainly lethal) effects are fairly well described, the short-term (i.e. sub-lethal) effects and cellular processes leading to cell death remain unclear. More specifically, the effects of allelochemicals on the biochemical composition of membranes, that seem to have a central role in allelochemical sensitivity [19] are unknown.

The aim of this study was to elucidate the mechanistic effects (at a subcellular level) of allelochemicals released by *Alexandrium minutum* on the common diatom *Chaetoceros muelleri* a diatom co-occurring with *A. minutum* [21]. The study focused on the photosynthetic apparatus and biochemistry of membranes. Allelochemicals were separated from *A. minutum* cells by filtration to specifically focus on allelochemical interactions and avoid interference by cell-cell interactions. A pulse of allelochemicals was performed to study the short-term sub-lethal effects of allelochemicals on *C. muelleri*, measuring the effects on photosynthesis, intracellular reactive oxygen species production and esterase activity, a proxy of primary metabolism [22,23]. To further explore the allelochemical interactions at the membrane level, we analyzed the membrane composition of the diatom (polar lipid classes, fatty acids composition of polar lipids, and sterol content) and pigments after 30 and 60 min of exposure to the filtrate. Neutral lipids classes were also analyzed to detect free fatty acid increases as a cell lysis indicator or a decrease of storage lipids (triacylglycerols) as a stress response.

2. Materials and methods

2.1. Microalgal cultures

A strain of *Alexandrium minutum* (strain CCMI1002, isolated from a bloom in Gearhies, Bantry Bay, Ireland [24]), not producing paralytic shellfish toxins (PST), was selected according to its high allelochemical potency [17]. The dinoflagellates were cultivated in triplicate for 10 days in 1 L of autoclaved L1 media [25] prepared with natural filtered (0.2 μm) seawater from Argenton, France (salinity = 34 psu, pH = 8.4) a pristine site for *Alexandrium* sp. Cultures of *A. minutum* were maintained in exponential growth phase to obtain a concentration of 9.6×10^4 cells mL^{-1} on the experimental day (growth rate: 0.18 day^{-1}). A strain of *Chaetoceros muelleri* (strain CCAP 1010-3 obtained from the CCAP culture collection, formerly listed as *Chaetoceros neogracile* or *Chaetoceros* sp.) was selected because of its sensitivity to *A. minutum* allelochemicals [10,17] and because of the ubiquity of its genus in phytoplankton communities [26]. Cultures of the diatom were grown for 10 days (2.12×10^6 cells mL^{-1}) in 6 L of autoclaved synthetic ocean seawater [27] enriched with L1 media (salinity = 35 psu, pH = 8.4) and silicate (1.06×10^{-4} M). Cultures of *C. muelleri* were in late exponential growth phase on the day of the experiment (growth rate: 0.15 day^{-1}). All cultures were maintained at 18 °C under a continuous light intensity of 100–110 μmol photons m^{-2} s^{-1}. Cultures were not axenic but were handled under sterile conditions to minimize additional bacterial contamination.

2.2. Exposure to allelochemicals

Allelochemicals were separated from cultures by centrifugation (10 min, 800*g*, 18 °C) followed by a filtration (0.2 μm, Minisart syringe filter, acetate cellulose membrane, 16534-K, Sartorius) of supernatant [10]. The filtrate concentrations were expressed as cell concentration (cells mL^{-1}) based on the initial culture concentration prior to filtration. Cultures of *C. muelleri* in late exponential growth phase were diluted to 1.38×10^6 cells mL^{-1} with filtered seawater and distributed to six 2 L glass balloon flasks. The diatom *C. muelleri* was then exposed in triplicate to two different conditions for 90 min: i) 12 mL of *A. minutum* filtrate (final concentration from 1200 *A. minutum* cells mL^{-1}), ii) the same volume (12 mL) of filtered seawater as a control (final volume in each condition 1 L). During the exposure (maximum 90 min), the flasks were kept under ambient laboratory light and temperature conditions (not measured).

2.3. Photosynthetic parameters

Photosynthetic measurements were performed by pulse amplitude modulation (PAM) fluorometry using an AquaPen-C AP-C 100 with a blue light (455 nm). Sub-samples of *C. muelleri* were dark-adapted for at least 10 min, allowing the full oxidation of photosystem II (PSII) reaction centers and electron chain transport, before the measurement of fluorescence variables and PSII maximum quantum yield ($Fv/Fm = (F_m - F_0) / F_m$). F_0 is the initial fluorescence intensity, and F_m the maximal intensity under saturating light [28]. Measurement of fluorescence variables were performed every 10 min for 100 min following the exposure.

Non-photochemical quenching (NPQ) measurements were performed in a separate experiment. Six replicates of the diatom *C. muelleri* (2×10^5 cells mL^{-1}) in exponential growth phase were exposed for 45 min to *A. minutum* filtrate (final *A. minutum* concentration of 6×10^3 cells mL^{-1}). The cells of *C. muelleri* were exposed to three successive flashes of 60 s (saturated light, 3000 μmol photons m^{-2} s^{-1}) and 3 dark phases of 80 s for NPQ measurements, in the presence of filtered seawater or filtrate.

2.4. Flow-cytometry measurements

Population growth counts and measurements of phytoplanktonic cell variables were performed using a FACScalibur (BD Biosciences, San Jose, CA, USA) flow cytometer with a 488 nm argon laser. Counts were estimated according to flowrate [29]. Cell variables, e.g. forward scatter (forward scatter, FSC), side scatter (side scatter, SSC) and red autofluorescence (Fl3, red emission filter long pass, 670 nm) were used to select diatom population. All flow cytometry measurements on *C. muelleri* (cell variables, esterase activity and reactive oxygen species production) were performed after 30, 60 and 90 min of exposure to *A. minutum* filtrate or filtered seawater.

Microalgal esterase activity, a proxy of primary metabolism was assessed with fluorescein diacetate staining (FDA; Invitrogen # F1303; at a final concentration of 6 μM) [22,23,30,31]. FDA is a nonpolar compound that can permeate into the cells. Once inside the cells, FDA is cleaved by esterases into acetate and fluorescein molecules (emission wavelength 525 nm), which are retained within the cells. Intracellular concentrations increase with both metabolic activity and time. Samples were incubated with the stain for precisely 10 min in the dark prior to flow-cytometry measurements. Intracellular fluorescence intensity, which is proportional to the amount of FDA cleaved by esterases within the cells, was measured with Fl1 emission filter (green emission filter band pass, 530/30 nm). Cell populations of *C. muelleri* could be divided into 3 sub-populations: cells with highest Fl1 fluorescence corresponding to high esterase activity (metabolically active), cells with a low Fl1 fluorescence corresponding to cells with reduced esterase activity (reduced metabolism), and cells which Fl1 fluorescence

Download English Version:

https://daneshyari.com/en/article/11032769

Download Persian Version:

https://daneshyari.com/article/11032769

Daneshyari.com