Harmful Algae 39 (2014) 259–270

Contents lists available at ScienceDirect

Harmful Algae

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

Characterization of spirolide producing *Alexandrium ostenfeldii* (Dinophyceae) from the western Arctic

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ARTICLE INFO

Article history:
Received 4 June 2014
Received in revised form 18 August 2014
Accepted 23 August 2014
Available online 14 September 2014

Keywords:
Alexandrium ostenfeldii
Greenland
Spirolides
Lytic activity

ABSTRACT

Toxin producing dinoflagellates of the genus *Alexandrium* Halim represent a risk to Arctic environments and economies. This study provides the first record and a characterization of *Alexandrium ostenfeldii* in the western Arctic. During a cruise along the coasts of western and southern Greenland 36 isolates of the species were established in August 2012. Plankton samples taken at three different stations from the upper water layer at water temperatures of approx. 4–7 °C, contained low amounts of *A. ostenfeldii*. Sequencing of SSU and ITS-LSU rDNA and subsequent phylogenetic analyses identified all Greenland strains as members of a NW Atlantic spirolide producing phylogenetic clade. Molecular results were confirmed by morphological features typical for this group (=Group 5 of a recent ITS-LSU phylogeny of *A. ostenfeldii*). The Greenland isolates did not contain either Paralytic Shellfish Poisoning toxins or gymnodimines, but produced several spirolides. Altogether 12 different analogs were detected, of which only SPX-1, C, 20-meG and H have been described earlier. The remaining 8 spirolides have not been identified so far. Some of them were found to dominate the toxin profiles of a number of isolates. Among the 36 investigated strains spirolide composition varied considerably, particularly isolates from western Greenland (Station 516) exhibited a high diversity of analogs, with different profiles in nearly all 22 isolates. All of the 34 tested Greenland strains showed considerable lytic capacity when exposed to *Rhodomonas salina*.

1. Introduction

World-wide, the majority of the toxic bloom-forming harmful algal species belong to the dinoflagellate genus *Alexandrium*. Species of *Alexandrium* are often globally distributed, occurring in a variety of habitats and all geographic zones (Taylor et al., 1995; Lilly et al., 2007). Many *Alexandrium* species are able to produce potent toxins, such as paralytic shellfish toxins (PSTs), which affect the neuromuscular, sensory, digestive and cardiovascular systems of human and other vertebrates (Hallegraeff, 1993; Selina et al., 2006) and account for most of the harmful events caused by members of the genus (Anderson et al., 2012). These algal toxins represent a serious risk for the environment and human health (Hallegraeff, 1993).

One of the less studied toxic species of the genus is *Alexandrium ostenfeldii*. It has been widely observed in temperate waters of Europe (Balech and Tangen, 1985), North America (Cembella et al., 2000a), the Russian Arctic (Okolodkov and Dodge, 1996) and Eastern Siberian Seas (Konovalova, 1991). There are also records of the occurrence of *A. ostenfeldii* from the coast of Spain (Fraga and Sanchez, 1985), the Mediterranean (Balech, 1995), New Zealand (Mackenzie et al., 1996), Peru (Sánchez et al., 2004) and Japan (Nagai et al., 2010). However, for a long time, *A. ostenfeldii* has been considered mainly as a background species, occurring at low cell concentrations mixed with other bloom forming dinoflagellates (Balech and Tangen, 1985; Moestrup and Hansen, 1988; John et al., 2003). Only in the past decade it has gained increasing attention when dense blooms of this species (or its synonym *A. peruvianum*) were reported e.g. from South America (Sánchez et al., 2004), the Northern Baltic Sea (Kremp et al., 2009), along the Adriatic coast of Italy (Ciminiello et al., 2006), the estuaries of the US East coast (Tomas et al., 2012), and, most recently, the Netherlands (Burson et al., 2014). It is not clear whether the recent increase in bloom

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http://dx.doi.org/10.1016/j.hal.2014.08.008
1568-9883/

events is due to anthropogenic spreading or changing environmental conditions favoring bloom formation. Most of the recent blooms occurred during summer in coastal areas and were associated with warm water periods (e.g. Hakanen et al., 2012). Experimental studies indicate that increased water temperature has a favorable effect on *A. ostenfeldii* bloom populations and it has been suggested that changing climate conditions promote bloom formation (Kremp et al., 2012). The species produces PSP toxins (Hansen et al., 1992), spirolides (Cembella et al., 2000a) and gymnodimines (Van Wagoner et al., 2011), and all compounds may even occur together in one strain (Tomas et al., 2012). Thus, an increase of *A. ostenfeldii* bloom events with several potent toxins involved may represent a new risk to the environment that is associated with climate change.

Most of the global *Alexandrium ostenfeldii* records are from cold-water environments and the species has long been considered to have an arctic-boreal distribution (Okolodkov, 2005). It was originally described from the north-east coast of Iceland (Paulsen, 1904), and has thereafter been reported mainly from high latitude waters of the North Atlantic (Cembella et al., 2000a; Brown et al., 2010), Scandinavia (Tangen, 1983; Moestrup and Hansen, 1988) as well as arctic and subarctic waters of northern Siberia and the Russian Far East (Konovalova, 1991; Okolodkov, 2005; Selina et al., 2006). In a recent study on *Alexandrium tamarense* in Greenland, the presence of *A. ostenfeldii* in the western Arctic was briefly mentioned (Baggesen et al., 2012).

It has been predicted that anthropogenic climate change is causing dramatic changes the Arctic area, including increased temperature (Screen and Simmonds, 2012) and rapid decline of glaciers, ice cover (Comiso et al., 2008), ice thickness (Kwok and Rothrock, 2009), and resulting in ice-free summer conditions in future. These changes will have large effects on many marine species including primary producers (Wassmann et al., 2008). Though the responses of the Arctic marine ecosystems to climate change are not well known, temperature increase has been considered one of the changes affecting the performance, abundance and distribution of arctic organisms most significantly (Alcaraz et al., 2014). Temperature increase and larger ice-free regions have, for example, been suggested to expand the distribution ranges of HAB-species into or within the Arctic sea-area (Hallegraeff, 2010) and cause severe problems to the sensitive Arctic environment due to toxin production, and their accumulation in higher trophic levels.

Since *Alexandrium ostenfeldii* is present in arctic and subarctic waters, it could be one of the first harmful dinoflagellate species to be favored by the increase of water temperature and the predicted cascading effects of climate change in the ecosystem (Walsh et al., 2011). Alternatively, populations from temperate coastal waters of the North Atlantic or Pacific area may expand their ranges and cause toxic blooms in the Arctic. Most of the recently reported *A. ostenfeldii* blooms are caused by representatives of a brackish, warm-water adapted globally distributed genotype (Tomas et al., 2012; Kremp et al., 2014). They differ from most of the other *A. ostenfeldii* isolates by their potential to produce PSP toxins in addition to or instead of spirolides and to potentially produce neurotoxic gymnodimines. This genotype has recently expanded within the northern Baltic Sea, a boreal cold-water system, presumably as result of increased summer surface temperatures (Kremp et al., 2009), and now regularly forms toxic blooms here. Most North Atlantic isolates, including subarctic strains from northern Iceland, though cluster in a different phylogenetic group and mainly produce spirolides. Spirolides are potent neurotoxins causing rapid death of mice when injected intraperitonally and are thus regarded as "emerging" toxins, even if the currently are not regarded as toxic to humans and therefore not regulated.

Despite abundant records of *Alexandrium ostenfeldii* from arctic coasts, arctic populations have not been characterized in terms of phylogenetic affiliation and important phenotypic traits such as morphology, toxicity and allelopathic potency. Such information is important for assessing the potential for bloom formation and risks of toxicity in a region where shellfish industry is an important part of the local economy (Garcia, 2006). Here we present molecular, morphological and physiological data of multiple *A. ostenfeldii* strains isolated from western and southern Greenland and provide the first, to our knowledge, extensive phylogenetic and morphological characterization as well as a detailed description of toxin profiles and lytic capacity of arctic populations of this species.

2. Materials and methods

2.1. Sampling and sample preparation

A total of 36 clonal strains of *Alexandrium ostenfeldii* were established from water samples collected at three stations at the west coast of Greenland (Fig. 1) during a cruise aboard the research vessel "Maria S. Merian" in August 2012. Vertical net tows were conducted at each station through the upper 30 m of the water column with a 20-μm-mesh Nitex plankton net. Total volume of each net tow concentrate was measured and a 20 mL subsample was fixed with paraformaldhyde (1% final concentration).

Seawater samples were taken at standard depths of 3, 8, and 20 m depth by means of 5 L Niskin entrapment bottles mounted on a remotely triggered rosette-sampler. 50 mL water samples were fixed with neutral Lugol (2% final concentration) in brown glass bottles.

2.2. Plankton composition

For a qualitative and quantitative characterization of the plankton community at the three stations where *Alexandrium ostenfeldii* were isolated, both net tow and bottle samples were

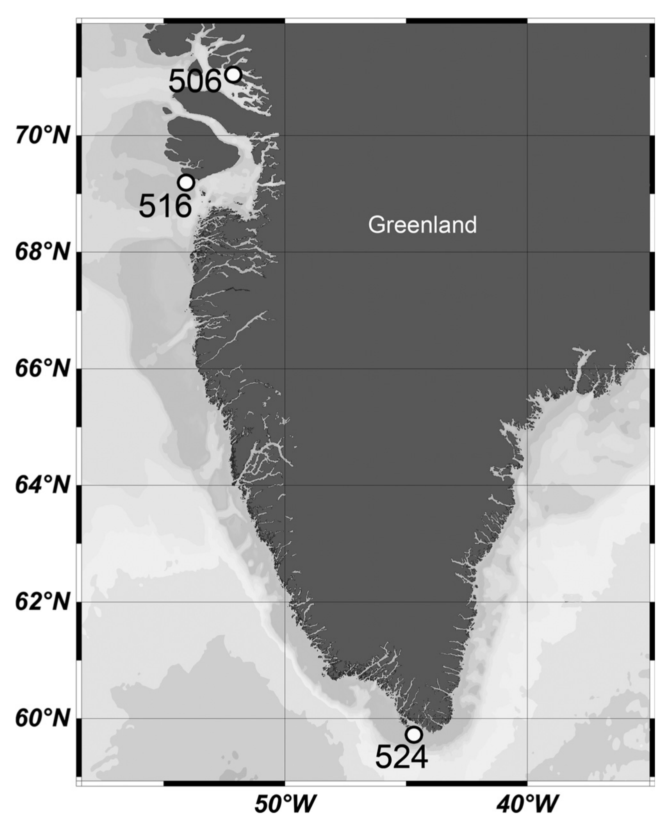

Fig. 1. Map of the southern part of Greenland with sampling stations at the western and southern coasts.