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Bioactive compounds of marine dinoflagellate isolates from western Greenland and their phylogenetic association within the genus *Alexandrium*

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ABSTRACT

The diversity and biogeography of populations of the toxigenic marine dinoflagellate genus *Alexandrium*, a major global cause of paralytic shellfish poisoning (PSP), are represented by only a few studies based upon a low number of cultured isolates and remain poorly described in Arctic and sub-Arctic waters. Multiple clonal isolates ($n = 22$) of the *Alexandrium tamarense* species complex, and a single isolate of *A. tamutum*, were collected from the water column while on board an oceanographic expedition to the west coast of Greenland. After culturing of these isolates under controlled conditions, their phylogenetic affinities within the genus *Alexandrium* were characterized by sequence analysis of nuclear large subunit (LSU) rDNA. Based upon morphological and molecular genetic criteria, all isolates of the *A. tamarense* species complex were consistent with membership in the Group I ribotype (previously known as the North American ribotype). Phenotypic signatures were also analyzed based upon their respective profiles of paralytic shellfish toxins (PST) and allelochemical interactions against a target cryptophyte *Rhodomonas*, as determined by lytic potency. All isolates conforming to the *A. tamarense* Group I produced PST, but no toxins were detected in *A. tamutum* P2E2. Unusually, only carbamoyl toxins were produced among the *A. tamarense* Group I isolates from Greenland; sulfocarbamoyl derivatives, generally present in *A. tamarense* population from other locations, including the Arctic, North Pacific and North Atlantic, were absent from all isolates. Allelochemical activity, causing cell lysis of *Rhodomonas*, but generally being unrelated to cellular PST, was expressed by all *A. tamarense* isolates and also by *A. tamutum*, but varied widely in potency. Comparison of the genotypic (rDNA) and phenotypic (PST profile, allelochemical activity) characteristics of Greenland isolates with those of other Arctic populations reveals a complex pattern of intra-specific diversity. Estimation of diversity relationships is problematic because of the distinct patterns of divergence and lack of evidence of linkage among the alternative biomarkers and morphology. Nevertheless, such studies are necessary as the basis for constructing hindcasting scenarios and predicting changes in *Alexandrium* species distribution in the Arctic from the regional to the global scale.

1. Introduction

The globally distributed marine dinophycean genus *Alexandrium* Halim forms harmful algal blooms (HABs) primarily in coastal temperate and sub-tropical waters throughout the world (Anderson et al., 2012). Among more than 30 morphologically defined species in this genus, the majority are known to include toxigenic members, capable of biosynthesizing a wide array of well-defined toxins (saxitoxins, spirolides and/or goniodomins). Furthermore, many *Alexandrium* strains also produce poorly characterized allelochemicals capable of lytic activity against target plankton cells (Tillmann and John, 2002; Tillmann et al., 2008).

The Arctic Ocean and adjacent coastal and sub-Arctic waters represent a relatively under-exploited frontier for discovery of the interactions and relationships within and among *Alexandrium* populations. This is inspite of the fact that several members of the genus *Alexandrium* have long been known from Arctic and sub-Arctic waters, e.g. *A. ostenfeldii* was first described from Iceland (Paulsen, 1904). Generally, a number of surveys have revealed the

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occurrence of several putatively toxigenic dinoflagellates (e.g., *Protoceratium reticulatum*, *Dinophysis* spp., *Alexandrium* spp.) in Arctic-boreal waters, but none were considered purely endemic to the Arctic (Okolodkov and Dodge, 1996) and this biogeographical association was further supported by distributional data from multiple ship expeditions in the Eurasian Arctic (Okolodkov, 2005). The genus *Alexandrium* is considered to be native to the Norwegian Sea, where it is occasionally associated with paralytic shellfish poisoning (PSP), along the Norwegian coast as far north as 71° N (Tangen and Dahl, 1993). In 2013, blooms of *Alexandrium* and a first identification of PSP toxins in blue mussels were reported from Icelandic waters (Burrell et al., 2013). Other northern records of *Alexandrium* are available from almost all arctic and subarctic regions, including the Gulf of Alaska (Taylor, 1984; Horner et al., 1997), the Greenland Sea northwest of Spitzbergen (Heimdal, 1983), the Northwestern Passage at Igloolik (Bursa, 1961), the Barents and White Sea (Ratkova and Wassmann, 2005), the Beaufort Sea (Niemi et al., 2011), the Bering Sea (Konovalova, 1993; Selina et al., 2006; Orlova et al., 2007), and the Chukchi Sea (Gu et al., 2013b; Natsuike et al., 2013). Just a few recent papers, however, have reported in detail about toxigenicity, toxin profile, and/or phylogenetic associations of *Alexandrium* in the Arctic (Baggesen et al., 2012; Gu et al., 2013b; Natsuike et al., 2013; Tillmann et al., 2014) and an integrated perspective is still lacking.

Since the original description (as *Gonyaulax tamarensis* Lebour) of a taxon now considered to belong to *Alexandrium* Halim emend Balech, the taxonomic affiliations at the inter- and intrageneric and intraspecific levels have been in a state of flux, with attendant controversies. Particularly within the *Alexandrium tamarense* species complex, comprising several morphologically defined species (i.e., *A. catenella*, *A. fundyense*, *A. tamarense*), and several closely related taxa formerly assigned to *Protogonyaulax* Taylor, this has led to frequent nomenclatural inconsistencies. Attempts to produce a consistent taxonomy involving separation of species within this group, based upon morphological features (width of apical pore plate, cellular dimensions, presence of a ventral pore) and phenotypic characteristics (toxin composition, bioluminescence) have been unsuccessful. Earlier molecular approaches to resolve *Alexandrium* species via electrophoretic patterns of isozymes (Cembella et al., 1988) have now been supplanted by comparisons of nucleic acid sequences, particularly of the large (LSU) and small (SSU) subunit rDNA (Scholin and Anderson, 1994; Scholin et al., 1994). Based upon rDNA phylogenetic relationships, clades of these cryptic species were originally defined and designated according to biogeographical distribution (e.g., North American, Mediterranean, etc. (Scholin et al., 1994; John et al., 2003)), but because of distributional overlap these were later given numerical clade designations (Groups I, II, III, IV, and V) (Lilly et al., 2007; Anderson et al., 2012). Recently, John et al. (2014) have proposed re-definition of these clades as discrete species on the basis of rDNA sequences and integration of morphological features with toxin production capacity, and including limited information on mating compatibility studies, i.e. the biological (or reproductive) species criterion.

In this context, the main objective of the present study was to thoroughly characterize Greenland isolates of *Alexandrium*. One aspect is to determine how the phylogenetic affinities determined by rDNA analysis of Arctic populations of *Alexandrium* fit within the genus. Moreover, phenotypic signatures based upon toxin profiles and allelochemical interactions provide an estimate of population-wide intra-specific diversity that can be compared with those of other geographical locations in order to assess biogeographical affinities or the distinctness of endemic populations. The long term perspective is to begin integrating morphological and molecular criteria with phenotypic expression of Artic populations to better understand the evolution of species, e.g. under scenarios of past climatic change. This in turn will form the basis for constructing hindcasting scenarios and predicting changes in *Alexandrium* species distribution from the regional to the global scale.

2. Materials and methods

2.1. Plankton sampling and preparation

A total of 45 clonal isolates of *Alexandrium* spp. were established from a water sample collected at station 516 (Fig. 1), one of several stations sampled along the west coast of Greenland during the ARCHEMHAB cruise aboard the research vessel *Maria S. Merian* in August 2012 (MSM21/3). Two vertical net tows with a 20-μm-mesh Nitex plankton net were conducted through the upper 30 m of the water column of each station. Total volume of the net tow concentrate was measured and an 18 mL subsample was fixed with paraformaldehyde (PFA) (1% final concentration) for qualitative and quantitative plankton identification. The rest of each net haul was sequentially filtered through Nitex meshes of 200, 50 and 20 μm by gravity filtration and split into aliquots for extraction of lipophilic and hydrophilic toxins. A defined aliquot of each suspended plankton size fraction was pelleted by centrifugation for subsequent PSP toxin analysis.

Seawater samples were taken at standard depths (3, 8, and 20 m) by means of 5 L Niskin entrapment bottles mounted on a remotely triggered rosette-sampler equipped with a CTD device

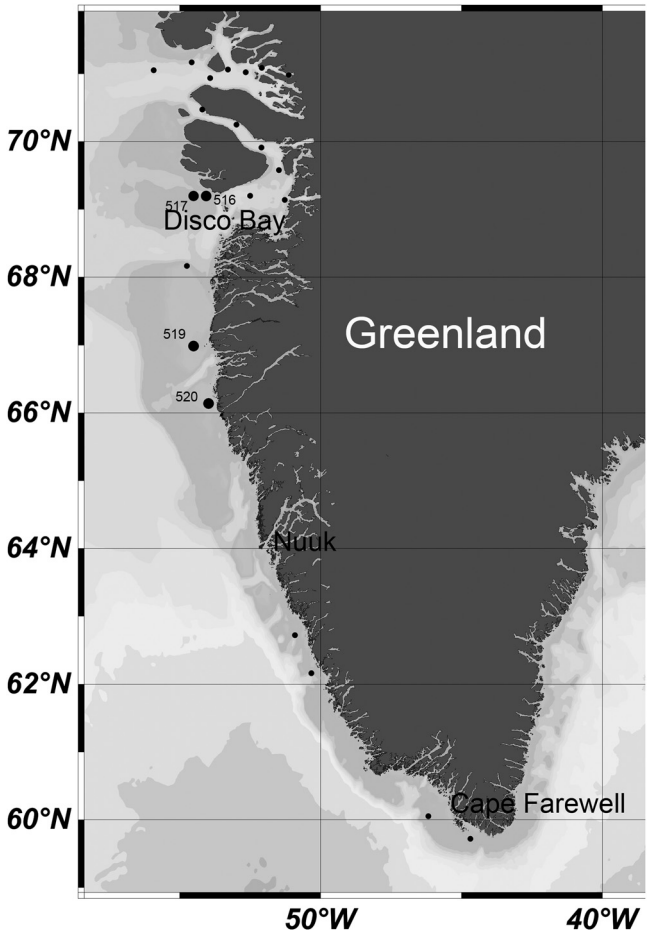

Fig. 1. Map of the sampling locations along the west coast of Greenland during the ARCHEMHAB cruise (Merian MSM21/3). Large dots with numbers indicate stations referred to in the text, small dots indicate additional sampling sites but where no PSP toxins were found in plankton samples.