Harmful Algae 51 (2016) 40–55

Contents lists available at ScienceDirect

Harmful Algae

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

Azadinium poporum from the Argentine Continental Shelf, Southwestern Atlantic, produces azaspiracid-2 and azaspiracid-2 phosphate

Urban Tillmann [a,*], C. Marcela Borel [b], Facundo Barrera [c], Rubén Lara [c], Bernd Krock [a], Gastón O. Almandoz [d], Matthias Witt [e], Nicole Trefault [f]

[a] Alfred Wegener Institute, Am Handelshafen 12, D-27570 Bremerhaven, Germany
[b] Instituto Geológico del Sur (CONICET – Universidad Nacional del Sur), Departamento de Geología, Laboratorio de Palinología, San Juan 670, 8000 Bahía Blanca, Argentina
[c] Instituto Argentino de Oceanografía, Biogeoquímica Marina, IADO – CONICET, Camino la Carrindanga km 7,5 c.c. 804, B8000FWB, Bahía Blanca, Argentina
[d] División Ficología, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Paseo del Bosque s/n, B1900FWA, La Plata, Argentina
[e] Bruker Daltonik GmbH, Fahrenheitstr. 4, 28359 Bremen, Germany
[f] Centro de Genómica y Bioinformática, Universidad Mayor, Camino La Pirámide 5750, Huechuraba, Santiago, Chile

ARTICLE INFO

Article history:
Received 5 August 2015
Received in revised form 2 November 2015
Accepted 2 November 2015
Available online 2 December 2015

Keywords:
Azadinium
Azaspiracids
Southwestern Atlantic
Argentina

ABSTRACT

The marine dinophycean genus *Azadinium* has been identified as the primary source of azaspiracids (AZA), a group of lipophilic phycotoxins known to accumulate in shellfish. Blooms of *Azadinium* in the southern Atlantic off Argentina have been described from the 1990s, but due to a lack of cultures, the diversity of South-Atlantic *Azadinium* has not yet been fully explored and their toxin production potential is completely unknown. During a spring 2010 research cruise covering the El Rincón (ER) estuarine system (North Patagonian coast, Argentina, Southwestern Atlantic) a search was conducted for the presence of *Azadinium*. Although neither *Azadinium* cells nor AZA in field plankton samples were detected, 10 clonal strains of *Azadinium poporum* were successfuly established by incubation of sediment samples. Argentinean *A. poporum* were more variable in size and shape than the type description but conformed to it by the presence of multiple pyrenoids with starch sheath, in plate pattern and arrangement, and in the position of the ventral pore located on the left side of the pore plate. In contrast to all previous description of *A. poporum*, isolates of the Argentinean *A. poporum* possessed a distinct field of pores on the second antapical plate. Conspecificity of the Argentinean isolates with *A. poporum* was confirmed by molecular phylogeny of concatenated ITS and LSU rDNA sequences, where all Argentinean isolates together with some Chinese *A. poporum* strains formed a well-supported ribotype clade within *A. poporum*. All isolates produced AZA with the same profile, consisting of AZA-2 as the major compound and, to a lesser extent, its phosphated form. This is the first report of a phosphated marine algal toxin. This first confirmation of the presence of AZA producing *Azadinium* in the Argentinean coastal area underlines the risk of AZA shellfish contamination episodes in the Southwestern Atlantic region.

1. Introduction

Harmful algal blooms and accumulation of phycotoxins in marine organisms pose a serious risk for human health (Glibert et al., 2005). Among the many known toxins of microalgal origin, azaspiracids (AZA) are a relatively new class of lipophilic compounds responsible for Azaspiracid Shellfish Poisoning (AZP). Azaspiracids were first recognized in the 1990s following an outbreak of human illness in the Netherlands that was associated with ingestion of blue mussels cultivated in Killary Harbour, Ireland (McMahon and Silke, 1996). The polyether toxins found to be present in this shellfish batch were subsequently identified and named azaspiracids (Satake et al., 1998; Nicolaou et al., 2006). Since then, AZA contamination of mussels above the European Union's regulatory level of 0.16 mg AZA per kg mussel meat has been a recurrent and major problem in Ireland (Salas et al., 2011). This serious situation of high AZA levels in shellfish in Ireland seem to be exceptional, but over the last 15 years, AZA have

* Corresponding author.
E-mail address: urban.tillmann@awi.de (U. Tillmann).

http://dx.doi.org/10.1016/j.hal.2015.11.001
1568-9883/

been documented in shellfish from many coastal regions of western Europe (James et al., 2002; Braña Magdalena et al., 2003; Furey et al., 2003; Amzil et al., 2008), northern Africa (Taleb et al., 2006; Elgarch et al., 2008), China (Yao et al., 2010), and North America (Trainer et al., 2013). In addition, AZA have been found in Japanese sponges (Ueoka et al., 2009) and Scandinavian crabs (Torgersen et al., 2008). Within the southern hemisphere, AZA have been detected in New Zealand (Smith et al., 2015) and there are reports from South America as well. Azaspiracids were found in Chile in shellfish (Álvarez et al., 2010; López-Rivera et al., 2010) and in plankton samples from the Pacific (Trefault et al., 2011). An *Azadinium*-like flagellate was recently reported by Proenca et al. (2014) from the south west Atlantic off Brazil, and the presence of low levels of AZA in shellfish has been recently detected in Argentinean waters (Turner and Goya, 2015).

Whereas the chemistry of the toxins and various aspects of the toxicology and pharmacology were quite well known (Hess et al., 2014; Twiner et al., 2014), the planktonic source remained elusive until in 2007 the small dinoflagellate, *Azadinium spinosum*, was unambiguously identified as a new species and as the primary source of AZA (Tillmann et al., 2009). Stimulated by this finding, a number of subsequent studies have revealed a high biodiversity of *Azadinium*, and a total of ten species are currently known (Tillmann et al., 2014a). Not all species of *Azadinium* produce toxins. Azaspiracids have been detected in the type species *A. spinosum* (Krock et al., 2009), in *A. dexteroporum* (Percopo et al., 2013), and new AZA have been detected in *Azadinium poporum* and in the closely related species *Amphidoma languida* (Krock et al., 2012), which together with *Azadinium* is combined in the family Amphidomataceae (Tillmann et al., 2012a).

All but one *Azadinium* species have been described from European waters (North Sea, North Atlantic, Mediterranean) (Tillmann et al., 2009, 2010, 2011, 2012b, 2014a; Percopo et al., 2013), the exception being *A. dalianense*, which has been described from the China Sea (Luo et al., 2013). Species of *Azadinium* are generally small, inconspicuous, and thus difficult to detect and to identify by regular light microscopy. Reliable records are thus based on the troublesome procedure of isolating, cultivating and fully characterizing local strains (in terms of morphology and molecular information) or are based on records of single specimens detected by electron microscopy of plankton samples. A compilation of all such available *Azadinium* records clearly shows that knowledge of the biogeography of the genus currently is rather limited and patchy, but nevertheless indicates that species of *Azadinium* have a global distribution (Tillmann et al., 2014c).

A low level of AZA in two shellfish samples from western South Atlantic was recently reported (Turner and Goya, 2015), supporting the notion that *Azadinium* is present in this area. In fact, a species of *Azadinium* had been described to form dense spring blooms in northern shelf waters off Argentina as early as 1990 and 1991 (Akselman and Negri, 2012), almost 15 year before the genus was erected. As shown by light microscopy (LM) and scanning electron microscopy (SEM) the species in question clearly had the *Azadinium* plate tabulation pattern and possessed a small antapical spine and was thus designated as *Azadinium* cf. *spinosum*. However, DNA samples and toxin measurements from these blooms were lacking, and for a final species designation, a few yet unresolved morphological details (e.g. presence of a ventral pore) of the Argentinean species needed to be clarified. Very recently, a retrospective description of a third bloom of the same species in 1998 was published (Akselman et al., 2014). Furthermore, additional cruise and time series data of the Argentinean Sea indicate a rather wide spatial distribution of *Azadinium* encompassing the northern Argentine and southern Uruguayan shelf including the mouth of Rio de la Plata (Akselman et al., 2014). Whereas the presence of *Azadinium* in Argentina is established, there is a lack of detailed morphological, molecular, and toxinological studies characterising the present species. It was thus the aim to use a research cruise of the R/V "Puerto Deseado" in 2010 to specifically search for the presence of *Azadinium* and to establish cultures for a detailed characterization.

Recurrent blooms of *Azadinium* in 1990, 1991, and 1998 (Akselman and Negri, 2012; Akselman et al., 2014) provided indications that cyst beds and cyst hatching may provide an important inoculum for local *Azadinium* populations. A number of dinoflagellates are known to produce cysts, mainly as a dormant, zygotic stage of their life cycle (Pfiester and Anderson, 1987). Such cysts can accumulate in the sediment, hatch after a dormant period and may thus act as "seed banks" with great ecological importance for bloom initiation. Knowledge on the life cycle and cyst formation of *Azadinium* is quite incomplete. Among species of *Azadinium*, cyst-like cells have been observed for only two species, *A. polongum* (Tillmann et al., 2012b) and *Azadinium poporum* (Gu et al., 2013). Successful isolation of *A. poporum* by incubating sediment samples (Potvin et al., 2012; Gu et al., 2013) is evidence for the presence of benthic resting stages in this species. Therefore, on the cruise sediment samples were taken in order to search for cysts of *Azadinium* and to use sediment in hatching experiments to obtain cultures of *Azadinium*.

The present study presents detailed morphological, molecular and toxinological characterization of ten isolates of *Azadinium poporum* obtained from incubating sediment samples from the El Rincón (ER) estuarine system, North Patagonian coast.

2. Materials and methods

2.1. Field campaign

2.1.1. Sampling

Samples were taken during a research cruise on board the R/V "Puerto Deseado" (CONICET-MINDEF, Argentina) during the austral spring in October 2010. The cruise covered the ER estuarine system, a shallow Frontal System of northern Patagonia, western South Atlantic, 39–42° S, 60–64° W of approximately 10,000 km^2 (Fig. 1). Most of the study area is shallow, the water depth at the sampling sites varying from 5 to 60 m. At all stations continuous profiles of temperature and salinity (CTD) were determined (Sea Bird model 911 plus with General Oceanic rosette; calibrated to a final precision 0.05 in salinity and 0.02 °C in temperature). Oceanographic data were obtained in collaboration with the Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP). Depth and off shore distance were also measured. Water samples were collected at 3 and 10 m depth from Niskin bottles. Aliquots of 100 mL of each depth were fixed with acidic Lugol's solution (1% final concentration) for qualitative and quantitative plankton analysis. For phycotoxin analysis, subsamples from Niskin bottles were pre-screened through a 20 μm mesh-size Nitex sieve and 1.5 L of the filtrate from each depth was mixed. Samples were filtered under gentle vacuum through 3 μm pore-size polycarbonate filters (Millipore, Eschborn, Germany). Filters were stored in 50 mL centrifuge tubes (Sarstedt, Nümbrecht, Germany) at −10 °C until analysis. Surface sediment samples were obtained by means of a pipette from the upper half centimeter, including the flocculent layer, of sediments collected by a Van Veen grab sampler during the Cruise at 21 stations. The samples were stored under nitrogen and sealed with parafilm in dark plastic bottles and kept at 4 °C to prevent cyst germination. Two sets of samples were obtained from the same grab sample of each station, one for the analysis of the organic-walled dinoflagellate cyst assemblages and the other for dinoflagellate cyst hatching experiments. The sediments selected for cysts hatching experiments were