



Genetic and toxinological characterization of North Atlantic strains of the dinoflagellate *Ostreopsis* and allelopathic interactions with toxic and non-toxic species from the genera *Prorocentrum*, *Coolia* and *Gambierdiscus*



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ABSTRACT

The genus *Ostreopsis* includes several toxic species that can develop blooms in benthic ecosystems, with potential harmful consequences for human health and marine invertebrates. Despite of this, little is known about the allelopathic interactions between these organisms and other co-occurring microalgae that exploit similar spatial and nutrient resources in benthic ecosystems. The aim of this study was to follow these interactions in cultures of two *Ostreopsis* ribotypes with different toxin profiles (*O. cf. ovata* contained ovatoxins-a, b, c and e, while only ovatoxin-d was found in *O. sp.* “Lanzarote-type”), mixed with species of three benthic dinoflagellate genera (*Coolia*, *Prorocentrum* and *Gambierdiscus*), isolated from the same area (North East Atlantic, Canary Islands). In a first experiment, the potential allelopathic effects on growth rates were followed, in mixed cultures of *Coolia monotis* (a non toxic species) exposed to the clarified medium and to cells of *O. sp.* “Lanzarote-type” and *O. cf. ovata*. Growth delayed in *C. monotis* was observed specially in clarified medium, while the *O. sp.* “Lanzarote-type” strain attained much lower densities in mixed cultures. In a second experiment, we examined the potential effects of clarified media from *O. sp.* “Lanzarote-type” and *O. cf. ovata* on the adherence capacity in two toxic species (*Prorocentrum hoffmannianum* and *Gambierdiscus excentricus*). Contrasting effects were found: a significant increase of adherence capacity in *P. hoffmannianum* vs attachment decline in *G. excentricus*, that experienced also severe deleterious effects (cell lysis). Our results suggest the existence of weak to moderate allelopathic interactions between the studied organisms, although the outcome is dependent on the species involved.

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1. Introduction

Harmful algal blooms (HABs) are natural toxic events which are –at times- related with increases of phytoplankton biomass and that represent a potential threat for the public health and/or aquatic ecosystems (Smayda, 1997). Dinoflagellates are one of the

most relevant HAB groups, and toxigenic species are sometimes, involved in this phenomenon (Liew et al., 2000).

In the recent decade, benthic and epiphytic dinoflagellates which inhabit sediments or live epiphytic on macroalgae-, have attracted much attention given the apparent geographical expansion of some toxic genera like *Ostreopsis* and *Gambierdiscus* (Berdalet et al., 2012; Shears and Ross, 2009). Dinoflagellate blooms are correlated with environmental changes in the water column (Pitcher et al., 2010), even so, in the benthos, the processes that shape dinoflagellate populations and facilitate the blooming of certain species are poorly understood (Fraga et al., 2012).

The genus *Ostreopsis* produces palytoxins (PLTXs) and palytoxin-like compounds (Ciminiello et al., 2011), such as ovatoxins (OVTXs), which are implicated in poisonings through ingestion of

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some clupeoid fishes, known as clupeotoxism (Onuma et al., 1999). In the Mediterranean Sea the blooms of *Ostreopsis* have been associated with skin irritations (Hoffmann et al., 2008) and irritative processes after inhalation of marine aerosols in the Tuscan and Ligurian coasts in Italy (Ciminiello et al., 2008; Totti et al., 2010), Andalusian (García et al., 2008) and Catalan coast in Spain (Vila et al., 2012). One of the most relevant episodes occurred in 2005, when about 200 people suffered respiratory illnesses provoked by ovatoxins and cells transported through the wind as marine aerosols as recently described by Ciminiello et al. (2014).

Although HABs like those of *Ostreopsis* can be nearly monospecific (Fraga et al., 2012; Nascimento et al., 2012), benthic communities of dinoflagellates are usually constituted by diverse taxa and species in different world areas. For example, the most widespread assemblages in NW Mediterranean Sea are composed of *Ostreopsis* sp., *Coolia monotis*, *Prorocentrum lima*, also in East Malaysia there have been usually reported associations of *Coolia*, *Amphidinium*, *Gambierdiscus*, *Ostreopsis* and *Prorocentrum* spp. (Vila et al., 2001; Tan et al., 2013). The co-occurrence of microalgae is conditioned by the balance between cell concentration (gains and losses), light and nutrient availability (Hutchinson, 1961). To overcome the low nutrients uptake, different adaptations have been proposed like nutrient retrieval through swimming, mixotrophy and allelopathic strategies (Smayda, 1997; Fraga et al., 2012).

The term “allelopathy” refers to the effect (positive or negative) produced in one population due to biochemicals secreted by a different one (Hulot and Huisman, 2004), and it can be understood as another adaptation (Legrand et al., 2003) that involves the secretion of biochemicals and the action of secondary metabolites (Arzul and Gentien, 2006). Distinct responses and sensitivity to allelochemical substances (Tillmann et al., 2008) could be mediated by specific donor/target combinations in each organism.

Toxic effects can be considered as allelopathic effects, but by no means are allelopathic effects restricted to toxins. There exist other molecules likely involved in allelopathy. Some alkaloids are able to inhibit RNA synthesis (Mendes and Vermelho, 2013) as well as other marine microorganisms can suppress β -glucosidase activity (Pandey et al., 2013) which is responsible for enzymatic activity. Allelopathy experiments are often carried out to test a hypothesis about interactions between target and donor species. Even though, other communities growing in non-axenic cultures (e.g. bacterial communities) could display feedback mechanisms and influence

target/donor species behaviour (Weissbach et al., 2010). Previous studies have documented different allelopathic interactions in planktonic dinoflagellates, such as cell death in *Karenia brevis* caused by *Skeletonema costatum* (Prince et al., 2008), growth inhibition in *Heterocapsa circularisquama* by *Karenia mikimotoi* (Uchida et al., 1999), cell lysis, morphological changes and motility loss in *Oxyrrhis marina* by *Alexandrium* spp. (Tillmann and John (2002), same for *Gymnodinium catenatum* exposed to the raphidophyte *Chattonella marina* (Fernández-Herrera et al., 2016), and protein activity inhibition when using supernatant from *Prorocentrum lima* which contains okadaic acid (Sugg and VanDolah, 1999). Allelopathy could be also implicated in favouring the growth of certain algae (Suikkanen et al., 2011), in mechanisms of predatory behaviour (Park and Kim, 2010), and in the initiation of their blooms by reducing growth of their competitors or affecting behavioural/physiological capabilities (Hakanen et al., 2014). In the case of benthic dinoflagellates, specifically the genus *Ostreopsis*, only a few reports have described weak to moderate allelopathic interactions between *O. cf. ovata* and other protists (Monti and Cecchin 2012; Pezolesi et al., 2011) or macroalgae (Accoroni et al., 2015).

The aim of the present work was to study the allelopathic interactions between *Ostreopsis* and several dinoflagellates that commonly co-occur in benthic assemblages, including observations on adherence capacity that have never been previously reported. For this purpose, a molecular and toxinological characterization of several *Ostreopsis* strains from the Canary Islands was performed. Consequently, two *Ostreopsis* ribotypes (*O. cf. ovata* and *O. sp.* “Lanzarote-type”) with different toxin profiles were selected to determine their effects (either by cell-to-cell contact or clarified medium) on cell viability, growth and adherence capacity, on toxic and non-toxic species of the genera *Coolia*, *Prorocentrum* and *Gambierdiscus* from the same geographical area.

2. Material and methods

2.1. Culture conditions

Experiments were conducted with clonal non-axenic strains (Table 1) from CCVIEO microalgal culture collection of the Instituto Español de Oceanografía (Vigo, Galicia, Spain). Cultures for mixed cells growth and supernatant experiments were grown in 250 ml

Table 1
Microalgal species used in the experiments, strain names, GenBank Accession numbers, isolation place, and isolation date.

Specie	Strain name	GenBank Accession No.	Isolation place	Isolation date
<i>Ostreopsis</i> sp. “Lanzarote-type”	VGO999	KP970827	^a Famara (Lanzarote)	Nov 2008
<i>Ostreopsis</i> sp. “Lanzarote-type”	VGO1000	KP970819	^a Famara (Lanzarote)	Nov 2008
<i>Ostreopsis cf. ovata</i>	VGO1001	KP970820	^a Famara (Lanzarote)	Nov 2008
<i>Ostreopsis</i> sp. “Lanzarote-type”	VGO1150	KP970825	^a Punta Hidalgo (Tenerife)	Sep 2009
<i>Ostreopsis</i> sp. “Lanzarote-type”	VGO1014	KP970821	^a Las Cabras (La Palma)	Mar 2010
<i>Ostreopsis</i> sp. “Lanzarote-type”	VGO1015	KP970822	^a Las Cabras (La Palma)	Mar 2010
<i>Ostreopsis</i> sp. “Lanzarote-type”	VGO1016	KP970823	^a Las Cabras (La Palma)	Mar 2010
<i>Ostreopsis cf. ovata</i>	VGO1017	KP970824	^a Las Cabras (La Palma)	Mar 2010
<i>Ostreopsis cf. siamensis</i>	VGO1187	KP970826	Tarifa (Cádiz, Spain)	Sep 2014
<i>Ostreopsis cf. ovata</i>	VGO1188	KP970813	^a Las Canteras (Gran Canaria)	2013
<i>Ostreopsis cf. ovata</i>	VGO1189	KP970814	^a Las Canteras (Gran Canaria)	2013
<i>Ostreopsis cf. ovata</i>	VGO1190	KP970817	^a Las Canteras (Gran Canaria)	2013
<i>Ostreopsis cf. ovata</i>	VGO1191	KP970818	^a Las Canteras (Gran Canaria)	2013
<i>Ostreopsis cf. ovata</i>	VGO1192	KP970816	^a Las Canteras (Gran Canaria)	2013
<i>Ostreopsis cf. ovata</i>	VGO1193	KP970815	^a Las Canteras (Gran Canaria)	2013
<i>Coolia monotis</i>	VGO858		^a Charco del Conde (La Gomera)	Nov 2005
<i>Prorocentrum hoffmannianum</i>	VGO1031	KT275812	^a La Puntilla (Gran Canaria)	Feb 2010
<i>Gambierdiscus excentricus</i>	VGO791		^a Punta Hidalgo (Tenerife)	Mar 2014

^a Canary Islands (Spain).

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