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Phylogenetic and functional diversity of bacterioplankton during *Alexandrium* spp. blooms

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Abstract

The phylogenetic and functional diversity of the bacterioplankton assemblage associated with blooms of toxic *Alexandrium* spp. was studied in three harbours of the NW Mediterranean. Denaturing gradient gel electrophoresis and DNA sequence analysis revealed the presence of a bacterium within the *Roseobacter* clade related to the presence of *Alexandrium* cells. Phylogenetic diversity was affected by the presence of *Alexandrium* spp., geographic situation and seasonality. In contrast, functional diversity, assessed with Biolog plates, was clearly affected by seasonality, but not by the presence of *Alexandrium*, indicating that the presence of the bacterium associated with the blooms was not enough to modify the metabolic pattern of the bacterioplankton assemblage.

Keywords: *Alexandrium*; Bacterioplankton; Phylogenetic diversity; Functional diversity; BIOLOG; DGGE

1. Introduction

Marine dinoflagellates of the genus *Alexandrium* include a number of species that produce paralytic shellfish poisoning toxins (PSTs). Blooms of toxic species of *Alexandrium* are common in coastal areas and occasionally cause losses to tourism, fisheries and aquaculture. Interactions between algae and bacteria are among the natural factors that might play a role in harmful algal bloom (HAB) dynamics [1]. Reported interactions of bacteria with *Alexandrium* species include promotion of ecdysis [2] and cyst formation [3], and algicidal effects [4]. The potential contribution of bacteria to *Alexandrium* spp. toxicity has been actively studied in the recent years, but is still a matter of debate [5–9]. It has been shown that Proteobacteria, particularly those of the *Roseobacter* clade, dominated the microbiota of *A. catenella* cultures [7] and, together with *Alteromonas*, of several other *Alexandrium* spp. cultures [10,11]. These interactions, observed in algal cultures, suggest a distinct composition of the bacterioplankton during HAB episodes. However, information on the composition of the bacterial assemblage during HABs is still very limited [12].

Changes in bacterioplankton structure may be important to carbon and nutrient flows, since the former is likely to affect functional diversity of the microbial community. Dinoflagellate blooms provide massive inputs of dissolved organic carbon (DOC) into coastal waters, triggering a response of the microbial community in cell number and activity [13]. However, in a study with algal cultures, Chen and Wangersky [14] observed that the DOC released from *A. tamarense* was not used by bacteria. Up to now, no information has been reported on

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bacterial utilization of carbon sources during *Alexandrium* spp. blooms.

Blooms of *Alexandrium* species are common in harbours along the NW Mediterranean [15]. We have studied phylogenetic and functional diversity of the bacterioplankton assemblage in three harbours of the Catalan coast, including two HAB episodes of *Alexandrium catenella* and *A. minutum*. In the present study, we address the following questions: (1) Is the phylogenetic composition of the bacterioplankton assemblage during HABs of the dinoflagellate *A. catenella* and *A. minutum* different compared to that in absence of HABs? (2) Is any species in the bacterioplankton assemblage reacting peculiar to harmful algal bloom situations? (3) Are the shifts in bacterial phylogenetic diversity associated with changes in functional diversity? Phylogenetic diversity was analyzed by denaturing gradient gel electrophoresis (DGGE) of 16S rRNA gene fragments. The most relevant bands in the gels were sequenced. Bacterial utilization of carbon sources was assessed by analyzing the community-level physiological profiles (CLPP) of the utilization of the 31 carbon sources in Biolog-ECO plates.

2. Materials and methods

2.1. Sampling and cultivation

Surface seawater was sampled from three harbours along the Catalan coast (NW Mediterranean). The Tarragona Harbour is located 100 km south of Barcelona and was sampled four times during a bloom of *A. catenella* in June 2001 and once in February 2003, when no toxic dinoflagellates were detected. The Arenys de Mar Harbour is located 40 km north of Barcelona and was sampled six times during a bloom of *A. minutum* in January–February 2002 and once in February 2003, when no toxic dinoflagellates were detected. Additionally, during 2001 and 2002, we took monthly samples from the Barcelona Harbour along two seasonal cycles in which toxic dinoflagellate blooms were not found. The Barcelona Harbour was used as a control of harbour without toxic dinoflagellates, and special attention was given to the periods coinciding with the bloom episodes in Arenys de Mar and Tarragona (June–July 2001 and January–February 2002). Phylogenetic analyses were performed on the bacterial assemblages of the harbour samples and on those found in the cultures of two dinoflagellate strains (*A. minutum* and *A. catenella*, kept in the culture collection of the Institut de Ciències del Mar). *A. minutum* was isolated from the Arenys de Mar Harbour in May 1996 and *A. catenella* from the Tarragona Harbour in June 1998. Cultures were grown in Guillards f/2 medium without silicate-enriched seawater, and maintained at 360–420 $\mu E\ m^{-2}\ s^{-1}$ (12 h light:-dark cycle) light intensity and a temperature of 25 °C. Carbon substrate utilization was determined by means of BIOLOG plates in the subset of samples shown in Table 1, which will constitute the basic data used in the present paper.

2.2. Environmental parameters

Chlorophyll *a* concentration was determined fluorimetrically according to Yentsch and Menzel [16]. A volume of 100 ml was filtered through Whatman GF/F filters. The filters were kept frozen at −20 °C for at least

Table 1
Properties of samples used to investigate differences in phylogenetic and functional diversity of bacterioplankton

Harbour	Date	TEMP	CHLa	BACT	TIN	SRP	N:P	ALEX
T	08 Jun 2001	22.8	367	1.5×10^{10}	21.6	1.07	20.2	3.20×10^{7}
T	12 Jun 2001	22.8	155	1.1×10^{10}	17.0	0.13	136.2	1.50×10^{7}
T	15 Jun 2001	22.3	17	8.1×10^{9}	11.8	0.21	56.0	2.70×10^{6}
T	19 Jun 2001	21.7	20	5.4×10^{9}	7.2	0.18	40.0	ND
A	18 Jan 2002	12.5	10.3	8.6×10^{8}	–	–	–	7.28×10^{5}
A	21 Jan 2002	12.5	–	1.1×10^{9}	–	–	–	7.25×10^{5}
A	29 Jan 2002	12.5	13.4	3.7×10^{9}	1.6	0.25	6.2	2.55×10^{6}
A	01 Feb 2002	12.3	13.8	1.5×10^{9}	2.2	0.34	6.7	1.45×10^{6}
A	05 Feb 2002	12.5	8.5	1.7×10^{9}	3.0	0.18	16.8	1.85×10^{6}
A	08 Feb 2002	12.6	4.7	1.5×10^{9}	2.3	0.15	15.3	2.15×10^{6}
B	05 Jun 2001	20.4	7.5	2.3×10^{9}	5.9	0.17	35.9	ND
B	15 Jun 2001	21.6	37.2	9.0×10^{9}	2.5	0.02	123.4	ND
B	20 Jun 2001	22.9	1.4	2.3×10^{9}	18.2	1.14	16.0	ND
B	12 Jul 2001	24.9	1.6	3.6×10^{9}	1.7	0.02	84.7	ND
B	16 Jan 2002	11.0	1.9	2.0×10^{9}	50.5	1.76	28.7	ND
B	30 Jan 2002	12.8	11.7	3.9×10^{9}	20.6	0.53	39.2	ND
B	14 Feb 2002	12.5	3.9	1.8×10^{9}	23.2	1.09	21.4	ND
B	28 Feb 2002	14.2	8.3	1.9×10^{9}	22.6	1.03	22.0	ND

TEMP: temperature, °C; CHLa: chlorophyll *a*, $\mu g\ l^{-1}$; BACT: bacterial concentration, cells l^{-1}; TIN: total inorganic nitrogen, μM; SRP: soluble reactive phosphorus, μM; N:P: TIN/SRP ratio; ALEX: *Alexandrium* abundance, cells l^{-1}. ND: not detected. (–): not available.