Helgol Mar Res (2004) 58:93–103
DOI 10.1007/s10152-004-0174-6

ORIGINAL ARTICLE

Antje Wichels · Christian Hummert · Malte Elbrächter · Bernd Luckas · Christian Schütt · Gunnar Gerdts

Bacterial diversity in toxic *Alexandrium tamarense* blooms off the Orkney Isles and the Firth of Forth

Received: 28 August 2003 / Revised: 26 February 2004 / Accepted: 1 March 2004 / Published online: 23 March 2004

Abstract The genetic diversity of the bacterial community associated with *Alexandrium tamarense* blooms was studied in blooms of the toxic dinoflagellates in the waters around the Orkney Isles and the Firth of Forth (Scotland). For toxin and molecular analysis of the bacterial communities associated with the toxic bloom, water samples were taken in 1998 and 1999 from *A. tamarense* blooms. The bacterial community structure, as determined by DGGE (denaturing gradient gel electrophoresis) showed clear differences between all three investigated size fractions (dinoflagellate-associated bacteria, attached bacteria and free-living bacteria), with high diversity within each sample. DNA sequence analysis of the dominant and most frequent DGGE bands revealed the dominance of α Proteobacteria, mainly of the *Roseobacter* clade, with similarities of 91–99%. Moreover, DGGE bands occurring at the same position in the gel throughout in most samples corroborate the presence of several specific α Proteobacteria of the *Roseobacter* clade. Overall, 500 bacteria were isolated from the bloom and partly phylogenetically analysed. They were members of two prokaryotic phyla, the Proteobacteria and the *Bacteroidetes*, related to Proteobacteria of the α and γ subdivisions (*Alteromonas*, *Pseudoalteromonas* and *Colwellia*). All bacteria were tested for the production of sodium channel blocking (SCB) toxins using mouse neuroblastoma assay. No production of SCB toxins was found and high performance liquid chromatography (HPLC) analysis confirmed these results. The content of total paralytic shellfish poisoning (PSP) toxin in the water samples, as measured within the toxic dinoflagellate blooms using HPLC, ranged from 53 to 2191 ng PSP l^{-1} in 1998 and from 0 to 478 ng PSP l^{-1} in 1999. Changes in PSP toxin content were not accompanied by changes of DGGE band patterns. We therefore presume that the bacterial groups identified in this study were not exclusively associated with toxic *A. tamarense*, but were generally associated with the phytoplankton.

Keywords Bacterial diversity · Toxic dinoflagellate bloom · Paralytic shellfish poisoning

Communicated by H.-D. Franke

A. Wichels (✉) · C. Schütt · G. Gerdts
Alfred-Wegener-Institut für Polar- und Meeresforschung,
Biologische Anstalt Helgoland,
27483 Helgoland, Germany
e-mail: awichels@awi-bremerhaven.de
Tel.: +49-4725-819245
Fax: +49-4725-819283

C. Hummert
Chemical and Biological Analyses,
WEJ GmbH,
21107 Hamburg, Germany

M. Elbrächter
Alfred-Wegener-Institut für Polar- und Meeresforschung,
Wadden Sea Station Sylt,
25992 List/Sylt, Germany

B. Luckas
Department of Biology and Pharmacy, Institute of Food Chemistry,
Friedrich-Schiller-University Jena,
07743 Jena, Germany

Introduction

The harmful algal bloom (HAB)-forming dinoflagellate *Alexandrium tamarense* produces potent neurotoxins called paralytic shellfish poisoning (PSP) toxins. When occurring in HABs, the toxin-producing *A. tamarense* can be accumulated in filter feeders, which in humans can lead to PSP upon consumption of these contaminated animals. Of the many natural factors thought to play an important role in HAB dynamics, algal-bacterial interactions are among the components increasingly cited as potentially important regulators of algal growth and toxicity (Doucette et al. 1998; Töbe et al. 2001). Up to now there have been some studies on bacteria associated with cultured toxic *A. tamarense* strains (Ogata et al. 1990; Franca et al. 1996; Gallagher et al. 1997; Doucette et al. 1998; Hold et al. 2001; Biegala et al. 2002). The bacteria were identified as Proteobacteria predominantly

related to the genera *Roseobacter*, *Alteromonas* and *Pseudomonas* (Kopp et al. 1997; Gallacher and Smith 1999). However, most investigations were performed with laboratory cultures and little is known about the situation in situ. Gallagher and Birkbek (1995) isolated bacteria during a toxic event at Ardtoe, Scotland and stated that 36.8% of the isolated bacteria produced sodium channel blocking (SCB) agents, as shown using mouse neuroblastoma (MNB) assay, but the study lacks any information about the identity of either the bacteria or the toxins. Until now, little information has been available concerning diversity and dynamics of bacterial populations within HABs. Furthermore, the role of specific bacterial groups identified in association with the toxic dinoflagellate is uncertain.

The aim of this study was to investigate the genetic diversity of the bacterial communities associated with water samples of different size fractions taken using denaturing gradient gel electrophoresis (DGGE) from toxic *A. tamarense* bloom events on the south-east Scottish coast and the Orkney Isles.

One objective was to describe the diversity of the bloom-associated bacterial communities by sampling a toxic bloom as it drifted with the water masses. In a second approach, a transect along the eastern Scottish coast covering about 425 km was sampled from the Firth of Forth to north-west of the Orkney Isles (Fig. 1). To identify specific bacterial groups, a comparative analysis of ribosomal genes was performed by partial sequencing of the 16S rDNA of bacterial isolates and excised DGGE bands. Quantification of PSP toxin content of the *Alexandrium* blooms, and determination of SCB toxin production of bacteria isolated from the toxic bloom should help to resolve the role of bacteria which are associated with toxic dinoflagellate blooms.

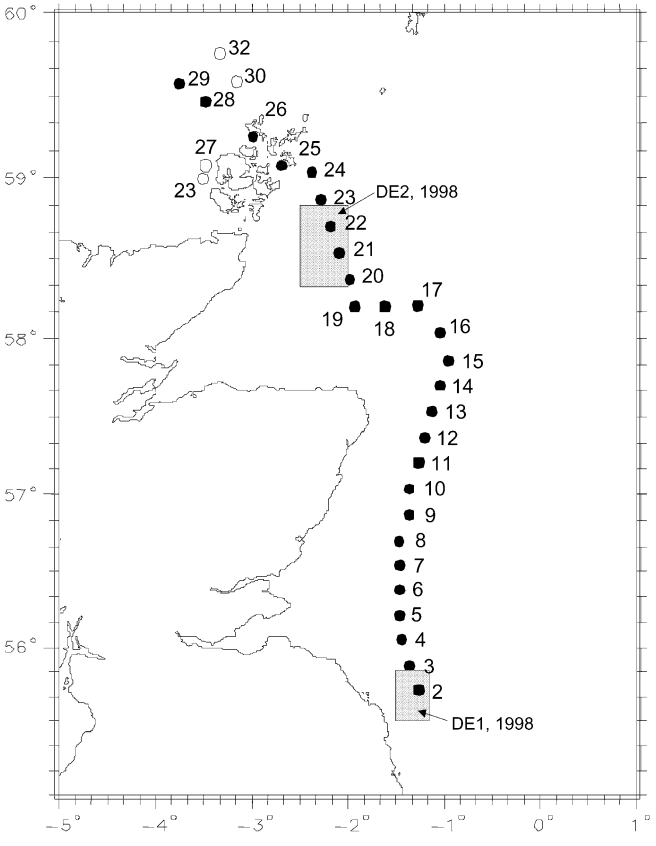

Fig. 1 Map of the east coast of Scotland and the North Sea displaying sampling stations of the research cruises in 1998 and 1999. *Grey boxes* indicate the area of two drift experiments DE1 and DE2 in 1998. In area DE1, 11 samples were taken. In area DE2, 15 samples were taken. *Filled circles* show stations sampled during a transect in 1999; *open circles* show stations sampled in 1998 in addition to the drift experiments

Methods

Sampling area and sampling strategy

Samples were taken in 1998 and 1999 from May to June during cruises with the research vessel "Heincke" off the eastern Scottish coast (Fig. 1).

In 1998, two drift experiments were performed, sampling the water body of a toxic *Alexandrium tamarense* bloom 11–15 times following a drifting buoy. The samples were taken in two areas, known as drift experiments 1 and 2 (DE1 and DE2; for sampling areas see Fig. 1). The area for DE1 was located south-east of the Firth of Forth, with 11 sampling stations. The area for DE2 was located south-east of the Orkney Isles, with 15 sampling stations. Additionally, four samples were taken outside the drifting area west and north-west of the Orkneys (Fig. 1, open circles). Prior to each drift experiment, phytoplankton samples were taken to detect *Alexandrium* cells by microscopic observations and to identify *A. tamarense* by toxin analysis (see below). The research vessel followed the drifting buoy and the course of the buoy was recorded. The drifting buoy was connected to four water sails of 5.2 m^2 arranged crosswise at 3–7 m water depth. Water samples were taken at depths of 2–5 m using a rosette sampler including a conductivity, temperature and depth probe. Oceanographic parameters (temperature, salinity and chlorophyll-*a* fluorescence) were recorded automatically.

In 1999, a transect along the eastern Scottish coast was sampled from the Firth of Forth to north-west of the Orkney Isles (Fig. 1, filled circles) covering about 425 km. A total of 29 stations were sampled with a peristaltic pump at depths of 1–3 m. The oceanographic parameters were recorded with the thermo-salinograph of the research vessel.

Abundance of *Alexandrium* sp.

To quantify the numbers of *Alexandrium* sp. cells, aliquots of the respective water samples were fixed with lugol solution and the numbers of *Alexandrium* sp. determined after the cruise using the Utermöhl method (Hasle 1978).

Sampling of bacterial biomass

Each water sample was processed by filtration in order to generate three fractions of bacterial biomass: (1) dinoflagellate-associated bacteria (>20 μm <100 μm); (2) attached bacteria (>3 μm <20 μm); and (3) free-living bacteria (>0.2 μm <3 μm). The filtration was performed as follows:

For the dinoflagellate-associated bacteria, the water sample (10 l) from each sampling station was prefiltered through 100-μm nylon screens to remove larger plankton organisms. The dinoflagellate biomass was concentrated onto 20-μm nylon gauze. To remove loosely attached bacteria, the biomass was washed ten

times with 100 ml sterile seawater containing cetyltrimethylammonium bromide (CTAB; 10 mg l^{-1}). The gauze was transferred to a sterile petri dish and dinoflagellate cells were washed off the screen with 1–2 ml STE buffer (6.7% sucrose w/v, 50 mM Tris-HCl, 1 mM EDTA, pH 8.0). The suspension was transferred to a reaction vial and stored at −20°C until analysis.

The bacterial biomass of the attached bacteria and free-living bacteria fractions were concentrated by pressure filtration. After prefiltration through 20-µm nylon screens, the water sample (5 l) was filtered onto a filter cascade (3 µm cellulose nitrate filter, Sartorius; followed by 0.22 µm Sterivex GS capsule filter, Millipore). The 3 µm filters were transferred to sterile petri dishes and frozen (−20°C). The capsule filters were rinsed with 10 ml STE, wrapped in parafilm and also frozen (−20°C).

Isolation of bacteria

Bacteria were isolated during both drift experiments on freshly prepared dinoflagellate extract medium as follows: to prepare the cultivation medium, plankton were sampled using a 20-µm net and prefiltered through 100-µm nylon mesh. Plankton smaller than 100-µm were collected in a second filtering step onto 20-µm nylon screens. The cells (mostly dinoflagellates as verified by microscopic observation) were transferred to 10 ml Nanopure water and sonicated 5 min on ice. Cell debris was removed by centrifugation (10,000 *g*). The dinoflagellate extract was filtered through 0.2 µm membranes to remove bacteria and added to 1 l sterile, filtered (<0.2 µm) seawater from the same station.

The bacteria associated with the HAB were harvested and inoculated as follows: at each sampling site, 10 l seawater was prefiltered through 100-µm nylon mesh gauze and the dinoflagellate cells were concentrated onto 20-µm nylon mesh gauze. Cells were washed as described before (see Sampling of bacterial biomass section) and finally broken up in 10 ml sterile seawater using an Ultra Turrax (20,000 rpm) for 5 min on ice. Dinoflagellate cell debris was removed by centrifugation (1,000 *g*) and the supernatant, which contained the bacteria, was used for inoculation of the dinoflagellate extract medium. After 2 weeks of incubation at in situ temperature (10°C) in the dark, aliquots were plated on marine agar medium 2216E with reduced nutrient concentration (2×10^{-3} of the original peptone and yeast-extract concentration) and incubated for 2–4 weeks. To obtain pure bacterial cultures, single colonies were picked from the agar plates and streak-plated at least twice on fresh agar plates. To gain sufficient biomass for DNA extractions and SCB testing, cultures were subsequently cultivated on high-nutrient media.

Sampling for toxins and toxin analysis

For toxin analysis from each station, water samples (3–5 l) were filtered onto glass fibre filters (Whatman GF/C). All samples were extracted with 1–2 ml 0.03 N acetic acid in reaction vials and sonicated for 1 min (60 W). Cellular debris and/or filter fragments were removed by centrifugation (12,000 *g*). Each supernatant was transferred to a new reaction vial and stored at −20°C until analysis by high performance liquid chromatography (HPLC, see below). Before HPLC analysis, extracts were passed through 0.45-µm nylon filters.

HPLC for toxin analysis was performed as published by Hummert et al. (1997). Toxin standards of STX, NeoSTX, GTX1, GTX2, GTX3 and GTX4 were purchased from the National Research Council Canada, Marine Analytical Chemistry Standards Program (NRC-PSP-1B), Halifax, NS, Canada; they also contained dcGTX2 and dcGTX3 as unquantified minor traces. Dc-STX was provided by the European Commission (BCR, The Community Bureau of Reference, Brussels). The PSP toxins were identified by comparing chromatograms obtained from sample extracts with those of the standard solutions containing GTX1 to GTX4, NeoSTX and STX. Quantification of the PSP contents was carried out comparing peak heights with corresponding calibration graphs (Hummert et al. 1997). Briefly, N-sulfocarbamoyl toxins are converted into the related carbamoyl toxins (B1 to STX, B2 to NeoSTX, C1 to GTX2, C2 to GTX3, C3 to GTX1 and C4 to GTX4) by hydrochloric acid treatment and quantified using the increase of peak levels in chromatograms resulting from extracts before and after hydrolysis.

MNB assay

The MNB assay for the detection of SCB toxins was performed according to the methods of Gallacher and Birkbeck (1992). Oubain and veratridine were added to MNB cells, leading to cell death due to sodium influx. In the presence of SCB toxins such as Saxitoxin, cells survived and were quantified by the addition of neutral red. The resultant colorimetric response was measured on a micro plate reader. All samples were tested in triplicate. The following conditions were used as controls: (1) six wells containing 200 µl RPMI tissue culture medium; (2) six wells containing oubain (1.5 mM) and veratridine (0.05 mM) adjusted to the final volume of 200 µl by the addition of material equivalent to the sample but containing no toxins (i.e. marine broth diluted 1/10 with RPMI medium for the test of supernatants, and 0.03 N acetic acid diluted 1/10 with RPMI medium for the test of extracts of bacterial biomass); (3) an STX dose response curve [STX (Calbiochem) diluted in RPMI medium] over a range of 0–400 nM in triplicate. The bacterial supernatants and extracts were used in the assay at a 10^{-1} dilution.

Nucleic acid extraction

Nucleic acid extraction was generally performed according to a modified protocol of Anderson and McKay (1983), omitting the NaOH step. Large, 3 µm filters were cut into pieces and the final extraction volume was increased to 10 ml. When filters displayed a brown colour, caused by humic acids, polyvinylpolypyrolidon (PVPP) was added (Holben et al. 1988). Membrane and capsule filters were incubated for 1 h at 50°C after addition of SDS.

All DNA extracts were kept in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) and served as template DNAs in the PCR. Prior to PCR amplification, the DNA extracts were analyzed by agarose gel electrophoresis on 0.8% agarose gels (1.5 h at 100 V in 0.5×TBE). After electrophoresis, gels were stained with ethidium bromide (0.5 mg l^{-1}). Photographs were taken on a UV transilluminator (302 nm) using Polaroid MP4 equipment (Cambridge, Mass.). The sample volume for subsequent PCR amplification was estimated from the photograph.

PCR conditions

Different regions of the 16S-rDNA were amplified by PCR. Table 1 displays the primer sequences and positions. For DGGE, a part of the 16S rDNA (V3 region) was amplified with the primers P2/P3 and P3/907R (Muyzer et al. 1993, 1995). Primers for amplification of the 16S rRNA gene of bacterial isolates were 8F/1408R (Lane 1991). PCR reactions with primer sets P2/P3, and P3/907R, and 8F/1408R (100 µl) mixture as follows: 0.2 µl DNA (capsule filter samples), 3 µl DNA (3-µm filter samples), 0.5–5 µl DNA (dinoflagellate cells), 0.2 µM each primer, 75 µM each dNTP, BSA (0.2 mg ml^{-1}) and 10 µl 10×PCR buffer [Gene Amp, Perkin Elmer; 100 mM Tris-HCl (pH 8.3), 500 mM KCl, 15 mM $MgCl_2$, 0.1% gelatin (w/v)]. The PCR amplification (Techne GENIUS, Cambridge) with primers P2/P3 and P3/907R was performed as described by Kirchner et al. (1999), modified by the following: after final primer extension (10 min), the temperature was lowered from 72°C to 50°C (1°C $cycle^{-1}$ min^{-1}) to prevent the formation of chimeric DNA (R. Söller, personal communication). PCR amplification with primers 8F/1408R was performed as described by Wichels et al. (1998). The amplification of PCR products of the appropriate size was confirmed by electrophoresis through a 1.4%

Table 1 Oligonucleotides used to amplify different rDNA regions

Primer	*Escherichia coli* position	Sequence	Reference
8f	8	5'-AGAGTTTGATCMTGGC	Lane (1991)
P3-clamp[a]	341	5'-CCTACGGGAGGCAGCAG	Muyzer et al. (1993)
P2	534	5'-ATTACCGCGGCTGCTGG	Muyzer et al. (1993)
907r	907	5'-CCGTCAATTCCTTTRAGTT	Muyzer et al. (1995)
1408r	1408	5'-GACGGGCGGTGTGTACAAG	Lane (1991)

[a] Clamp at the 5' end not shown

(w/v) agarose gel in TBE buffer, followed by staining with ethidium bromide (see above). For DNA sequencing, proper PCR products were purified through QIAquick spin columns (Qiagen).

Denaturing gradient gel electrophoresis

DGGE was performed using a Bio-Rad DCode system. PCR products of P2/3 were applied on 6% (w/v) polyacrylamide (Appligene) gels in 0.5× TAE buffer (20 mM Tris, 10 mM acetate, 0.5 mM EDTA) with denaturing gradients of 15–55% urea/formamide (100% denaturant contains 7 M urea and 40% formamide). Electrophoresis was run at 60°C and 140 V for 3 h. The denaturing gradient for the larger fragments (550 bp) amplified with P3/907R was 15–70%, electrophoresis was run at 60°C and 100 V for 17 h. After electrophoresis, the gels were removed from the glass plates, soaked for 15 min in nanopure water containing ethidium bromide (0.5 mg l^{-1}), rinsed in nanopure water overnight (12 h), and photographed under UV transillumination (302 nm) using Polaroid MP4 equipment (Cambridge, Mass.). Photographs were electronically digitized using a Linotype Saphir Ultra2 scanner and Adobe Photoshop software. DGGE bands (P3/907R) were excised from the DGGE gel, eluted (Sambrook et al. 1989) and reamplified with primers P3 (without clamp) and 907r (Muyzer et al. 1995). The reamplified PCR fragments were purified (QIAquick spin column, Qiagen) and stored until DNA sequencing at −20°C.

DNA sequencing and comparative sequence analysis

DNA sequencing of DGGE bands and bacterial isolates was done according to the manufacturer's instructions on a Liqor DNA 4200 sequencer using the SequiTherm EXEL II long read sequencing Kit-LC (Biozym). SequiTherm EXEL II DNA polymerase was added according to the instructions of the manufacturer (Biozym). Sequencing primers were 8F-IR, 798F-IR, 907R-IR and 1408R-IR for the bacterial isolates, and P3-IR (without clamp) and 907r-IR for the reamplified DGGE bands.

Sequence comparison

All sequences were aligned to those obtained from the Ribosomal Database Project (Maidak et al. 1997) or GenBank (Benson et al. 1997). Sequence alignment was performed with the sequence editor of CLUSTALW version 1.7 (Thompson et al. 1994).

Nucleotide sequence accession numbers

The sequences obtained for bacterial isolates are available from GenBank under accession numbers AY277258–AY277275.

Results

Oceanographic characteristics and PSP levels

In 1998, oceanographic characteristics were different between the two drift experiments DE1 and DE2 (Fig. 1). During DE1 east of the Orkney Isles, the buoy drifted for 56 h on a north-westerly course from 55°41′N 1°18′W to 55°33′N 1°24′W; the water was thermally stratified with a thermocline at about 15 m. Surface water temperature ranged from 9.32 to 10.45°C. Salinity ranged from 34.06 to 34.24 (Fig. 2B). During DE2, the buoy drifted for 93 h on a north-easterly course from 58°47′N 2°21′W to 55°33′N 2°12′W; almost no stratification could be observed in the water column. The surface water temperature ranged from 9.16 to 9.58°C and salinity from 34.898 to 35.059 (Fig. 2D).

Phytoplankton analysis was only performed in 1998. The toxic dinoflagellate *A. tamarense* occurred in all samples from both drift experiments in concentrations ranging from 660 to 1,600 N l^{-1} during DE1 (Fig. 2A) and 920 to 5,380 N l^{-1} during DE2 (Fig. 2C).

For calculation of PSP levels, the sum of all PSP toxins was used. At most sampling stations from DE1 and DE2, the toxin levels followed the levels of *A. tamarense*. In detail, during DE1, 53 to 514 ng ΣPSP l^{-1} was found and during DE2, 575 to 2,191 ng ΣPSP l^{-1}.

In 1999, a larger area covering about 425 km along the Scottish coast was sampled displaying a broader range of temperature (8.42 to 9.81°C) and salinity (34.475 to 35.230; Fig. 2F). No quantitative phytoplankton analysis was done on samples from this transect. Hence, the results of the chemical analysis for PSP toxins represent the occurrence of toxic *Alexandrium* species indirectly. In samples from the transect, elevated PSP levels could only be detected from stations 14 to 18 north-east of Long Forties (Fig. 2E), ranging from 116 to 478 ng ΣPSP l^{-1}. Furthermore, in the area of the former drift experiments (1998), much lower PSP concentrations were detected on average, with 28–40 ng ΣPSP l^{-1} at stations 2 and 3 (i.e. DE1), and 0–182 ng ΣPSP l^{-1} at stations 20 to 23 (i.e. DE2). No PSP was detected in the area of the Orkney Isles.

DGGE analysis of microbial communities

The diversity of the microbial communities from the three sample fractions (>20 μm <100 μm dinoflagellate-asso-

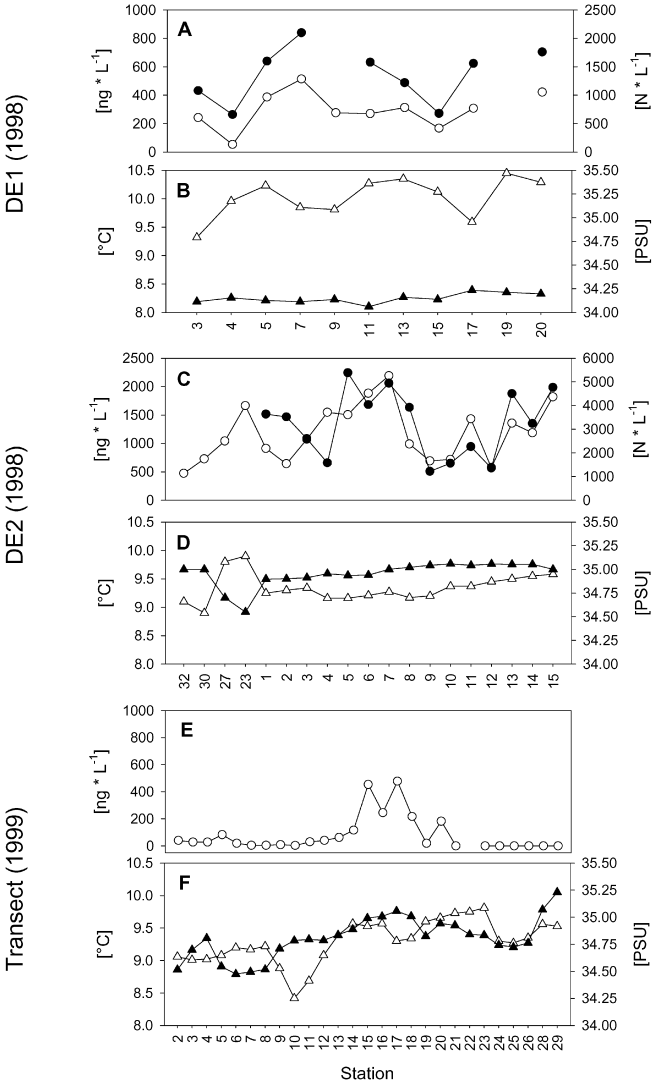

Fig. 2A–F Oceanographic parameters, total saxitoxin concentration and numbers of *Alexandrium tamarense* at sampling stations from the research cruises in 1998 and 1999. **A**, **C**, **E** *Open circles* show Saxitoxin concentration of particulate matter; *filled circles* show numbers of toxic dinoflagellate *A. tamarense*. **B**, **D**, **F** *Open triangles* show water temperature, *filled triangles* show salinity.

ciated bacteria, >3 μm <20 μm attached bacteria and >0.2 μm <3 μm free-living bacteria) was analyzed by DGGE of partial 16S rDNA fragments obtained by PCR amplification. DGGE analysis from samples of the drift experiments was performed using DNA fragments amplified using the primers P2/P3. DGGE analysis from the samples of the transect, collected in 1999, was performed using DNA fragments amplified with the primers P3/907R, resulting in larger DNA fragments. After DGGE, the larger fragments were analyzed by sequencing single DGGE bands (see below).

The band pattern obtained from samples of the two drift experiments revealed a diversity of 10 to 16 OTUs (operational taxonomic units) in the different size fractions (Fig. 3; attached bacteria fraction). Differences were observed between DGGE patterns of the two drift areas (Fig. 3). DGGE profiles from samples taken outside the drift areas (Fig. 3; stations 23, 27, 30 and 32) were also different from those of the drift experiments. Almost no differences were observed in band patterns between single sampling stations of each drift experiment (Fig. 3), but each fraction (>20 μm <100 μm dinoflagellate-associated bacteria, >3 μm <20 μm attached bacteria and >0.2 μm <3 μm free-living bacteria) displayed unique band patterns (data not shown).

The DGGE band patterns obtained from samples taken on a transect covering about 425 km along the eastern Scottish coast from the Firth of Forth to north-west of the Orkney Isles were also diverse, and all three size fractions were different (Fig. 4). Further differences occurred within the three sampling fractions, with small variations in band patterns in the free-living fraction (Fig. 4A) and higher variations in samples of the attached and the dinoflagellate fraction (Fig. 4B, C).

DNA sequence analysis

DNA sequence analysis of DGGE bands was performed with samples taken during the cruise. The main DGGE fragments of three DNA fractions were analyzed after excision and reamplification by DNA sequencing. From 36 bands excised from the DGGE gels, 22 were success-

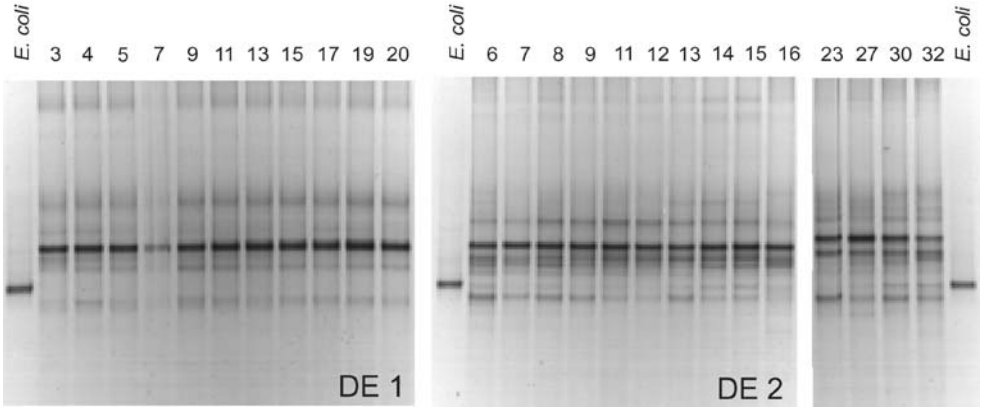

Fig. 3 DGGE profiles of 16S rDNA amplified fragments (200 bp) obtained from the size fraction of attached bacteria (size fraction >3 μm <20 μm) within toxic *A. tamarense* blooms south-east of the Firth of Forth and off the Orkney Isles

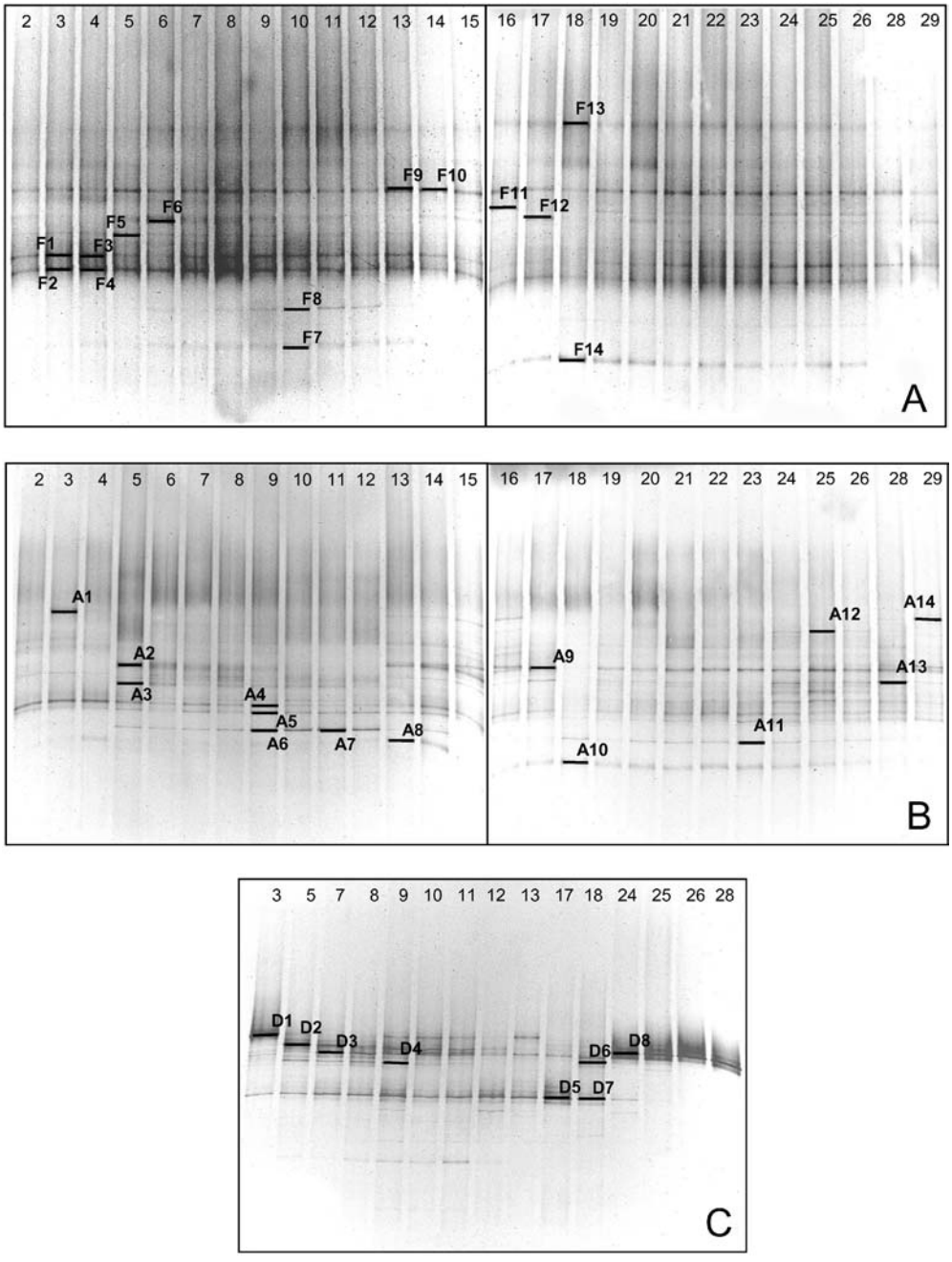

Fig. 4A–C DGGE profiles of 16S rDNA amplified fragments (500 bp) obtained from different size fractions within a toxic *A. tamarense* bloom south-east of the Firth of Forth and off the Orkney Isles. Extracted bands were DNA sequenced and are numbered. The relationships between excised band sequences and other sequences in GenBank are indicated in Table 3. **A** Free-living bacteria fraction (>0.2 μm <3 μm). **B** Attached bacteria fraction (>3 μm <20 μm). **C** Dinoflagellate-associated fraction (>20 μm)

fully reamplified and sequenced (eight bands from the free-living bacteria fraction; eight bands from the attached bacteria fraction and six bands from the dinoflagellate-associated bacteria fraction; Fig. 4). Most of the DGGE bands obtained from the free-living and attached bacteria fractions (12 bands) were related to α subdivision Proteobacteria of the *Roseobacter* clade, with similarities of 91–99% (Table 2). A single band had the best match to a sequence obtained from an excised DGGE band, related to an *A. tamarense* bloom with a low similarity value (88%) and was only distantly related to the α subdivision. Sequences of two co-migrating bands from the free-living bacteria fraction (F9, F10) were related to the *Bacteroidetes* phylum with similarities of 94%. One band of the attached bacterial fraction was similar to plastid DNA from the diatom *Skeletonema pseudocostatum* with 98% similarity. All six DGGE fragments obtained from the dinoflagellate-associated fraction showed sequence similarities to eukaryotic DNA, chloroplasts or plastids from diatoms or other eukaryotes with similarities of 96–99%. Sequences recovered from co-migrating bands from the different sampling stations were highly identical (e.g. F1/F3, A6/A7; Table 3), and the slight differences observed were assumed to be artefacts. Only two co-migrating bands (F9/F10; Fig. 4) were less similar, which might be due to the poor sequence quality of the recovered DGGE

Table 2 Phylogenetic affiliation of bacteria isolated from the dinoflagellate biomass fraction of a toxic *Alexandrium tamarense* bloom sampled in 1998 during drift experiment 2. The station numbers refer to the number of the sampling point within the drift experiment

Station	Isolate	Best match (accession no.); similarity (%)	No. nucleotides compared	Division	Group
3	H11	*Pseudoalteromonas tetraodonis* (X82139); 99%	1,080	γ Proteobacteria	*Pseudoalteromonas*
	H12	*Pseudoalteromonas* sp. ICO13 (U85859); 99%	960	γ Proteobacteria	*Pseudoalteromonas*
	H13	*Pseudoalteromonas* sp. ICO13 (U85859); 98%	900	γ Proteobacteria	*Pseudoalteromonas*
	H14	Gamma Proteobacterium UMB7C (AF505732); 97%	810	γ Proteobacteria	*Alteromonas*
	H15	*Pseudoalteromonas* sp. ICO13 (U85859); 98%	900	γ Proteobacteria	*Pseudoalteromonas*
	H22	*Pseudoalteromonas* sp. H120 (AF069667); 98%	840	γ Proteobacteria	*Pseudoalteromonas*
	H92	*P. carrageenovora* (X82136); 97%	890	γ Proteobacteria	*Pseudoalteromonas*
12	H3	*Microscilla arenaria* (AB078078); 93%	840	*Bacteroidetes*	*Flexibacter*
	H24	*Sulfitobacter* sp. LM-11 (AJ534236); 98%	915	α Proteobacteria	*Rhodobacteria*
	H25	*Sulfitobacter* sp. LM-11 (AJ534236); 98%	900	α Proteobacteria	*Rhodobacteria*
	H2	*Hyphomicrobium indicum* (AB016982); 97%	840	γ Proteobacteria	*Vibrio*
	H4	*P. nigrifaciens* (X82146); 98%	880	γ Proteobacteria	*Pseudoalteromonas*
	H16	*Vibrio anguillarum* (ssp. H1 11431) (X71821); 98%	800	γ Proteobacteria	*Vibrio*
	H17	*Pseudoalteromonas* sp. ICO13 (U85859); 98%	925	γ Proteobacteria	*Pseudoalteromonas*
	H18	*Pseudoalteromonas* sp. ICO13 (U85859); 99%	900	γ Proteobacteria	*Pseudoalteromonas*
	H21	*Pseudoalteromonas* sp. H120 (AF069667); 98%	1,200	γ Proteobacteria	*Pseudoalteromonas*
	H23	*Glaciecola* sp. HA02 (AB049729); 98%	940	γ Proteobacteria	*Alteromonas*
	H94	*Glaciecola* sp. HA02 (AB049729); 97%	860	γ Proteobacteria	*Alteromonas*

band F10. Some co-migrating DGGE bands could be detected in samples from several stations (Fig. 4). These are, for example, F2, F9, A6 and A9. For the last of these DGGE bands, no DNA sequence could be determined.

Eighteen bacterial strains were isolated in 1998 from the dinoflagellate fraction, as described above, and were phylogenetically analysed. Fifteen bacterial isolates belong to γ subdivision Proteobacteria (Fig. 3). Most of them (10 isolates) were related to the *Pseudoalteromonas* group with similarity values of 96–99%, three isolates were similar to bacteria from the *Alteromonas* group (97–98% similarity), and two were related to *Vibrionaceae* (98% similarity). Two bacterial isolates were closely related to α subdivision Proteobacteria of the *Rhodobacter* clade with similarity values of 98%. One strain was distantly related to *Microscilla arenaria* from the *Bacteroidetes* phylum (similarity value 93%).

MNB assay

Overall, 50 bacterial strains were isolated and analyzed for SCB toxin production. All bacteria tested (supernatants and cell extracts) were SCB toxin negative. For 18 bacterial strains, this negative result was also confirmed by HPLC analysis.

Discussion

The characterization of multiple 16S rRNA-defined populations by DGGE and DNA sequencing provides a useful tool for the analysis of bacterial diversity within toxic *A. tamarense* blooms off the eastern Scottish coast. The toxic algal bloom was characterized by microscopic counts of *A. tamarense* and by PSP toxin analysis. Furthermore, bacterial isolates obtained from the toxic dinoflagellate biomass fraction were tested for SCB activity, which might indicate PSP toxin production, and were in part analysed for their phylogenetic affiliation.

The bacterial community structure, as determined by DGGE band patterns during toxic *A. tamarense* blooms, revealed high diversity within all investigated sample fractions (>20 μm <100 μm dinoflagellate-associated bacteria, >3 μm <20 μm attached bacteria and >0.2 μm <3 μm free-living bacteria). For the drift experiments DE1 and DE2, general differences between DGGE patterns were observed, but within a single drift experiment uniform band patterns were found within a single size fraction. The two drift experiments were characterized by differences in temperature and salinity, with higher temperature and lower salinity during DE1 than DE2, which indicates the sampling of different water bodies. Therefore, it is assumed that sampling of different water bodies might be one reason for the variation in the bacterial community structure between DE1 and DE2.

Differences were also observed among size fractions for all of the samples: this has already been reported in a number of studies, indicating that attached bacteria are indeed phylogenetically different from free-living bacteria (DeLong et al. 1993; Acinas et al. 1997; Crump et al. 1999; Riemann et al. 1999; Fandino et al. 2001). In the Mediterranean Sea, size fractions were found to be different in terms of rDNA internal spacer analysis (Acinas et al. 1999), while variations within each size fraction from the same depth (i.e. free-living versus attached bacteria) was found to be relatively small (Acinas et al. 1997). In surface waters, the diversity of picoplankton was relatively constant, even at a large scale (offshore versus costal waters) (Acinas et al. 1997). However, the uniformity of band patterns in all three size fractions obtained from samples from the drift experiments was much higher than those obtained on a transect from the south-east Scottish coast to south-east of the

Table 3 Phylogenetic relationship between reamplified and sequenced DGGE bands isolated from different fractions of a toxic *A. tamarense* bloom off the Orkney Isles, sampled in 1999

DGGE band	Best match (accession no.); similarity (%)	Most closely related sequence from validly described bacterial species (accession no.); similarity (%)	Division/group
F1[a]	*Ophiolis aculeata* symbiont (U63548); 96%	*Ruegeria atlantica* (D8526); 92%	α Proteobacteria, *Roseobacter* group
F2[a]	*O. aculeata* symbiont (U63548); 98%	*R. gelatinovorans* (D8523); 94%	α Proteobacteria, *Roseobacter* group
F3[a]	*O. aculeata* symbiont (U63548); 96%	*R. atlantica* (D8526); 92%	α Proteobacteria, *Roseobacter* group
F4[a]	*O. aculeata* symbiont (U63548); 98%	*R. gelatinovorans* (D8523); 94%	α Proteobacteria, *Roseobacter* group
F5[a]	Uncultured α Proteobacterium (AF466884); 88%	*Ahrensia kieliense* (D88524); 81%	Proteobacteria
F8[a]	Uncultured α Proteobacterium Shippagan (AF100168); 91%	*Staleya guttiformis* (Y16427); 90%	α Proteobacteria, *Roseobacter* group
F9[a]	Uncultured Cytophagales (AY033305); 94%	*Gelidibacter algens* (AF001367); 90%	*Bacteroidetes*; *Flavobacteria*
F10[a]	Uncultured Cytophagales (AY033305); 94%	*G. algens* (AF001367); 90%	*Bacteroidetes*; *Flavobacteria*
A4[b]	Uncultured *Roseobacter* NAC-11-3 (AF245632); 93%	*R. gelatinovorans* (D8523); 93%	α Proteobacteria, *Roseobacter* group
A5[b]	Uncultured marine bacterium D015 (AF177555); 97%	*R. algicola* (X78315); 97%	α Proteobacteria, *Roseobacter* group
A6[b]	Uncultured marine bacterium D015 (AF177555); 98%	*R. algicola* (X78315); 97%	α Proteobacteria, *Roseobacter* group
A7[b]	Uncultured marine bacterium D015 (AF177555); 98%	*R. algicola* (X78315); 97%	α Proteobacteria, *Roseobacter* group
A8[b]	Uncultured α Proteobacterium Shippagan (AF100168); 99%	*Staleya guttiformis* (Y16427); 97%	α Proteobacteria, *Roseobacter* group
A10[b]	Uncultured α Proteobacterium Shippagan (AF100168); 99%	*Staleya guttiformis* (Y16427); 97%	α Proteobacteria, *Roseobacter* group
A11[b]	*Crassostrea virginica* symbiont CV1010-362 (AF114485); 96%	*R. gelatinovorans* (D8523); 95%	α Proteobacteria, *Roseobacter* group
A14[b]	*Skeletonema pseudocostatum* 16S rRNA (X82155); 98%		Diatoms
D1[c]	*Skeletonema pseudocostatum* 16S rRNA (X82155); 98%		Diatoms
D2[c]	*Skeletonema pseudocostatum* 16S rRNA (X82155); 96%		Diatoms
D3[c]	Unidentified picoplankton clone OM20 (U32670); 98%	*Skeletonema pseudocostatum* 16S rRNA (X82155); 96%	Eukaryota, unclassified plastid
D4[c]	Uncultured vent bacterium ML-2d (AF208994); 96%	*Skeletonema pseudocostatum* 16S rRNA (X82155); 96%	Eukaryota, unclassified plastid
D5[c]	Environ. clone OCS20 chloroplast gene (AF001654); 99%	*Guillardia theta* plastid (AF041468); 96%	Eukaryota, unclassified plastid
D8[c]	*Skeletonema pseudocostatum* 16S rRNA (X82155); 97%		Diatoms

[a] Free-living bacteria
[b] Attached bacteria
[c] Dinoflagellate-associated bacteria

Orkney Isles. This is particularly true for the attached bacteria and the dinoflagellate-associated fractions. However, as shown by DNA sequence analysis, the uniform band patterns of the dinoflagellate biomass fraction to some extent contained PCR fragments of eukaryotic DNA from plastids (see Results). This fraction has to be considered with caution regarding both the uniformity of band patterns and the bacterial diversity (i.e. number of OTUs), which were probably lower than observed.

Fandino et al. (2001) hypothesized that changes in bacterial species composition are related to changes in bacterial community metabolism, and may thus be due to differences in organic material available or higher variations in nutrients in different parts of the bloom, possibly coupled with different growth stages of the phytoplankton. The authors showed that remarkable shifts of bacterial community structure in a dinoflagellate bloom were correlated with chlorophyll-*a* content and ectoenzymatic activity. In our study, the variation in band patterns in attached bacterial fraction samples taken on the transect in 1999 was not correlated with PSP toxin concentration. Since remarkable changes were found in temperature and salinity at the different stations, the significant changes in bacterial community structure is more likely to be related to changes in these oceanographic parameters than to the occurrence of toxic dinoflagellates.

Overall, the phylogenetic diversity of bacteria, as identified by DGGE band sequencing and by sequencing of bacterial isolates, was limited to two bacterial phyla,

the Proteobacteria and *Bacteroidetes*, with the former restricted to the α and γ Proteobacteria subdivisions. This has also been shown for bacteria associated with toxic and non-toxic dinoflagellate species in culture (Hold et al. 2001), in samples from a Scottish PSP monitoring site by fluorescent in situ hybridization (Töbe et al. 2001), and also in non-toxic phytoplankton blooms (Cottrell and Kirchman 2000b). Additionally, the dinoflagellate-associated fraction was composed of sequences similar to 16S ribosomal genes from the plastids of eukaryotic algae. This has previously been observed in a diatom mesocosm study and in a dinoflagellate bloom by Riemann et al. (2000) and Fandino et al. (2001). These authors reported that, as with other molecular approaches for culture-independent studies on microbial ecology, DGGE-based analysis suffers from inherent problems. They suggested careful analysis of the methodology at each step in order to limit the effect of artefacts. In this case, biases like PCR primer specificity may cause amplification of plastid or cyanobacterial genes, and thus may lead to an overestimation of the diversity of the bacterial community. Since the DGGE fragments with eukaryotic origin were always found exclusively in the dinoflagellate-associated fraction, the bacterial diversity within this fraction was difficult to estimate. However, the other two fractions were unaffected. PCR-DGGE analysis showed that the free-living and attached bacteria fractions from the toxic *A. tamarense* bloom were dominated by α Proteobacteria. This was also confirmed by a special DGGE for α Proteobacteria (data not shown). Within the α Proteobacteria, sequences were mostly related to the *Roseobacter* clade. Since samples were taken from depths of 3–7 m, these results support those of Acinas et al. (1999), who also found high diversity in the free-living bacteria fraction with a dominance of α Proteobacteria near the surface. Also, in non-toxic algal blooms, the bacterioplankton is dominated by α Proteobacteria of the genus *Roseobacter* (Gonzales et al. 2000; Zubkov et al. 2001). In these studies, the phylotype *Roseobacter* was positively correlated with chlorophyll-*a* and DMSP concentrations.

Another significant group is bacteria of the *Bacteroidetes* phylum, a group which is often thought to be involved in particle degradation (De Long et al. 1993; Cottrell and Kirchmann 2000a; Riemann et al. 2000; Eilers et al. 2001; Fandino et al. 2001). Hold et al. (2001) also found bacteria from the *Bacteroidetes* phylum in *A. tamarense* cultures by DGGE band sequencing and by isolation. However, these sequences were only distantly related to sequences obtained in this study. DGGE bands, which appear to run at the same position in the DGGE gel, were shown to be identical (no. F9/F10, no. A6/A7; Fig. 4). Thus, it was possible to show one species of the group *Cytophaga* (no. F9) and one of the *Roseobacter* clade (no. F1, F2) to be present in all samples from the free-living fraction. Additionally, OTUs related with the *Roseobacter* clade (A4, A5, A6, A11) were present in all samples from the attached bacteria fraction. As already shown by Cottrell and Kirchmann (2000b), these two groups of bacteria play an important role in particle degradation, but no phylogenetic group dominated the consumption of all dissolved organic matter. Based on substrate uptake studies using micro-autoradiography, the authors suggested that the participation of a diverse assemblage of the three main groups, α and γ Proteobacteria and the *Cytophaga-Flavobacter* cluster, is essential for the complete degradation of complex DOM in the oceans.

Interestingly, the bacteria isolated from the dinoflagellate-associated fraction belong to these three known culturable groups: the *Roseobacter* clade; the *Pseudoalteromonas* group; and the *Alteromonas* group. A single strain was related to the *Bacteroidetes* phylum. Most of these isolates are related to species already described as being associated with dinoflagellates (Gallacher et al. 1997; Kopp et al. 1997; Töbe et al. 2001) or as being associated with the particle fraction in several marine environments (DeLong et al. 1993; Eilers et al. 2000). DNA sequences related to those from the *Pseudoalteromonas* and *Alteromonas* groups were found only in sequence data sets obtained from bacterial isolates (Fig. 5). This might be due to the isolation and cultivation strategy used in this study. Since bacteria were grown on a bacteria-free, dinoflagellate biomass extract, bacteria which are involved in degrading dinoflagellate cells were probably selected. Eilers et al. (2000) showed that the majority of bacterial isolates were members of the genera *Pseudoalteromonas*, *Alteromonas* and *Vibrio*; nevertheless, they constitute only a minor fraction of the bacterial community. Interestingly, their study revealed that these culturable γ Proteobacteria were often attached to particles and showed high cellular RNA contents. However, they could never be detected in situ in high numbers. From other studies it is known that several marine *Pseudoalteromonas* isolates have strong algicidal effects on harmful marine microalgae (Lovejoy et al. 1998). These bacteria may be involved in the termination of a bloom and can be interpreted as infectious. On the other hand, several bacteria from the *Alteromonas* group which have been isolated from toxic *A. tamarense* have been shown to be putatively toxic (Gallacher et al. 1997). In contrast, none of our isolates were capable of producing any of the PSP toxins. Furthermore, none of our previously isolated bacteria (300 strains) were positive with regard to PSP toxins (as confirmed by HPLC analysis, unpublished data). However, Hold et al. (2001) also found *Alteromonas*-related species in both toxic and non-toxic *Alexandrium* sp. strains in cultures. Furthermore, *Alteromonas*-related species have also been found in the Mediterranean Sea and the North Sea (Acinas et al. 1999; Kirchner et al. 1999; Seibold et al. 2001). They may somehow interact with *Alexandrium* sp. and may be involved in biotransformation of toxins (Gallacher and Smith 1999) or other compounds released by the algal cells.

These culturable bacterial groups of *Pseudoalteromonas* and *Alteromonas* species mostly belong to the fast-growing bacteria, which have been shown to be

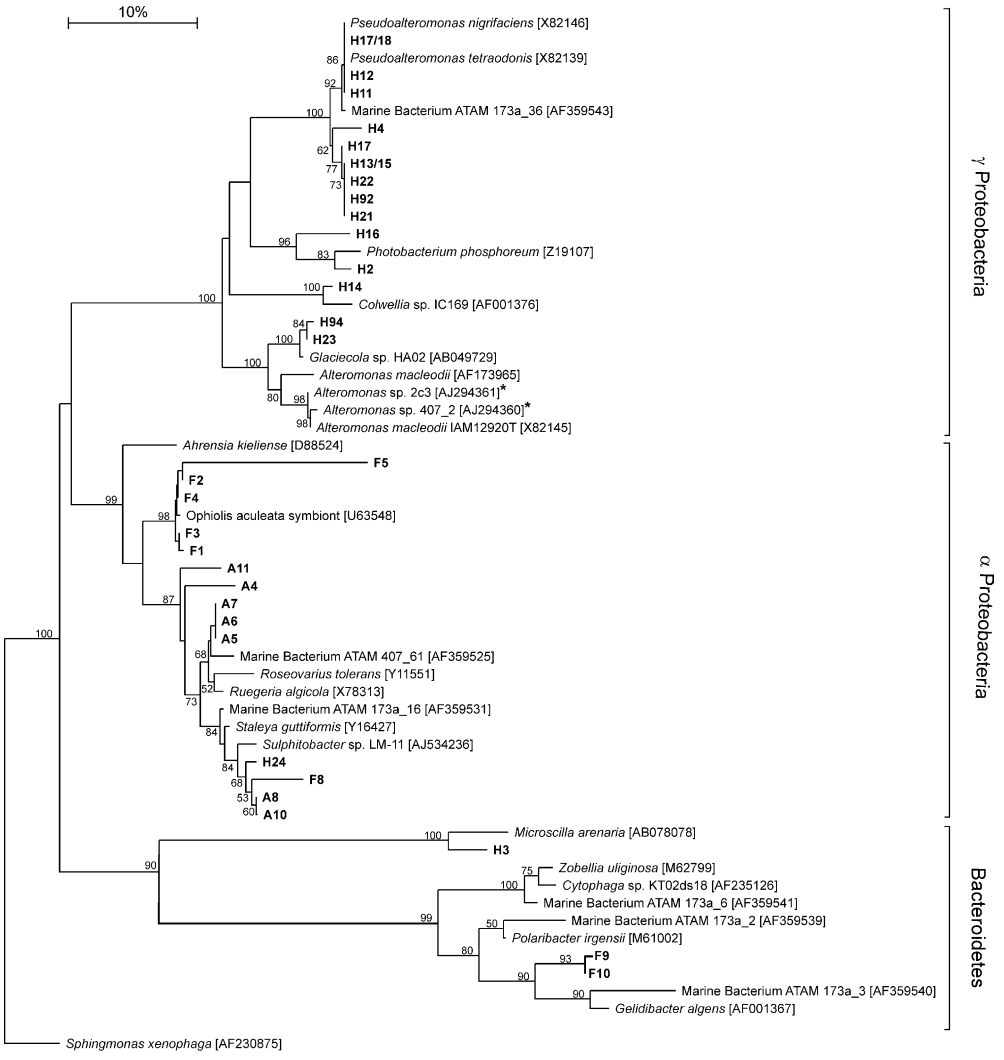

Fig. 5 Phylogenetic reconstruction using 16S rRNA genes, placing bacterial isolates and DGGE-generated DNA sequences from a toxic *A. tamarense* bloom and toxic *A. tamarense* species in culture (Hold et al. 2001) near their closest neighbours. Distance tree based on 400 bp of 16S rRNA. The bootstrap values equal to or greater than 50% are shown. * Putatively toxic bacterial isolates (Gallacher et al. 1997; Hold et al. 2001)

associated with dinoflagellates in culture and in the field, and are considered to be infectious. It might be hypothesized that these bacterial groups are possibly involved in the termination of a phytoplankton bloom and may function as a "disease". In contrast, non-culturable bacteria may represent the "satellite bacteria" (Schäfer et al. 2002), which are involved in the development of a bloom, or in the biotransformation of toxins, nutrients or other compounds released by the algae.

Acknowledgements This research was part of the BMBF project TEPS (03F0161A, B). We are very grateful to the crew of the RV 'Heincke'. We would like to thank Katrin Erler and Hilke Döpke for their excellent technical assistance.

References

Acinas SG, Rodríguez-Valera F, Pedrós-Alió C (1997) Spatial and temporal variation in marine bacterioplankton diversity as shown by RFLP fingerprinting of PCR amplified 16S rDNA. FEMS Microb Ecol 24:27–40

Acinas SG, Antón J, Rodríguez-Valera F (1999) Diversity of free-living and attached bacteria in offshore western Mediterranean waters as depicted by analysis of genes encoding 16S rRNA. Appl Environ Microbiol 65:514–522

Anderson DG, McKay LL (1983) Simple and rapid method for isolating large plasmid DNA from lactic Streptococci. Appl Environ Microbiol 46:549–552

Benson DA, Boguski MS, Lipman DJ, Ostell J (1997) GenBank. Nucleic Acids Res 25:1–6

Biegala I, Kennaway G, Alverca Elsa LJF, Vaulot D, Simon N (2002) Identification of bacteria associated with dinoflagellates (Dinphyceae) *Alexandrium* spp. using tyramid signal amplification—fluorescent in situ hybridisation and confocal microscopy. J Phycol 38:404–411

Cottrell MT, Kirchman DL (2000a) Community composition of marine bacterioplankton determined by 16S rRNA gene clone libraries and fluorescence in situ hybridization. Appl Environ Microbiol 66:5116–5122

Cottrell MT, Kirchman DL (2000b) Natural assemblages of marine proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter. Appl Environ Microbiol 66:1692–1697

Crump BC, Armbrust EV, Baross JA (1999) Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia river, its estuary, and the adjacent coastal ocean. Appl Environ Microbiol 65:3192–3204

DeLong EF, Franks DG, Alldrege AL (1993) Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. Limnol Oceanogr 38:924–934

Doucette GJ, Kodama M, Franca S, Gallacher S (1998) Bacterial interactions with harmful algal bloom species: bloom ecology, toxigenesis, and cytology. In: Anderson DM, Cembella AD, Hallegraeff GM (eds) Physiological ecology of harmful algal blooms. NATO ASI Series, vol G. Springer, Berlin Heidelberg New York, 41:619–647

Eilers H, Pernthaler J, Glöckner FO, Amann R (2000) Culturability and in situ abundance of pelagic bacteria from the North Sea. Appl Envir Microbiol 66:3044–3051

Eilers H, Pernthaler J, Peplies J, Glöckner FO, Gerdts G, Amann R (2001) Isolation of novel pelagic bacteria from the German Bight and their seasonal contributions to surface picoplankton. Appl Environ Microbiol 67:5134–5142

Fandino LB, Riemann L, Steward GF, Long RA, Azam F (2001) Variations in bacterial community structure during a dinoflagellate bloom analyzed by DGGE and 16S rRNA sequencing. Aquat Microb Ecol 23:119–130

Franca S, Pinto L, Alvito P, Sousa I, Vasconcelos V, Doucette GJ (1996) Studies on prokaryotes associated with PSP producing dinoflagellates. In: Yasumoto T, Oshima Y, Fukuyo Y (eds) Harmful and toxic algal blooms. International Oceanographic Commission in UNESCO, pp 347–350

Gallacher S, Birkbeck TH (1992) A tissue culture assay for direct detection of sodium channel blocking toxins in bacterial culture supernates. FEMS Microbiol Lett 92:101–107

Gallacher S, Birkbeck TH (1995) Isolation of marine bacteria producing sodium channel blocking toxins and the seasonal variation in their frequency in sea water. In: Lassus P, Arzul G, Ehard E, Gentien P, Marcaillou C (eds) Harmful marine algal blooms. Lavoisier, Intercept, pp 445–449

Gallacher S, Smith EA (1999) Bacteria and paralytic shellfish toxins. Protist 150:245–255

Gallacher S, Flynn KJ, Franco JM, Brueggemann EE, Hines HB (1997) Evidence for production of paralytic shellfish toxins by bacteria associated with *Alexandrium* spp. (Dinophyta) in culture. Appl Environ Microbiol 63:239–245

Gonzalez JM, Simó R, Massana R, Covert JS, Casamayor EO, Pedrós-Alió C, Moran MA (2000) Bacterial community structure associated with a dimethylsulfoniopropionate-producing North Atlantic algal bloom. Appl Environ Microbiol 66:4237–4246

Hasle GR (1978) Using the inverted microscope. In: Sournia A (ed) Phytoplankton manual. UNESCO Monogr Oceanogr Methodol 6:191–196

Holben WE, Jansson JK, Chelm BK, Tiedje JM (1988) DNA probe method for the detection of specific microorganisms in the soil bacterial community. Appl Environ Microbiol 54:703–711

Hold GL, Smith EA, Rappe MS, Maas EW, Moore ERB, Stroempl CSJR, Prosser JI, Birkbeck TH, Gallacher S (2001) Characterization of bacterial communities associated with toxic and non-toxic dinoflagellates: *Alexandrium* spp. and *Scrippsiella trochoidea*. FEMS Microbiol Ecol 37:161–173

Hummert C, Riutscher M, Reinhardt K, Luckas B (1997) Analysis of characteristic PSP profiles produced by *Pyrodinium bahamense* and several strains of *Alexandrium* using HPLC based on ion-pair chromatographic separation, post column oxidation and fluorescence detection. Chromatographia 45:312–316

Kirchner M, Sahling G, Schütt C, Döpke H, Uhlig G (1999) Intracellular bacteria in the red tide-forming heterotrophic dinoflagellate *Noctiluca scintillans*. Arch Hydrobiol Spec Issues Adv Limnol 54:297–310

Kopp M, Doucette GJ, Kodama M, Gerdts G, Schütt C, Medlin LK (1997) Phylogenetic analysis of selected toxic and non-toxic bacterial strains isolated from the toxic dinoflagellate *Alexandrium tamarense*. FEMS Microbiol Ecol 24:252–257

Lane DJ (1991) 16S/23SrRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) Nucleic acid techniques in bacterial systematics. Wiley, New York, pp 115–175

Lovejoy C, Bowman JP, Hallegraeff GM (1998) Algicidal effects of a novel marine *Pseudoalteromonas* isolate (class Proteobacteria, gamma subdivision) on harmful algal bloom species of the *Chattonella*, *Gymnodinium*, and *Heterosigma*. Appl Environ Microbiol 64:2806–2813

Maidak BL, Olsen GJ, Larsen N, Overbeek R, McCaughey MJ, Woese CR (1997) The RDP (Ribosomal Database Projekt). Nucleic Acids Res 25:109–110

Muyzer G, Waal de EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 59:695–700

Muyzer G, Hottenträger S, Teske A, Wawer C (1995) Denaturing gradient gel electrophoresis of PCR-amplified 16S rDNA—a new molecular approach to analyze the genetic diversity of mixed microbial communities. Mol Microb Ecol Manual 3.4.4, pp 1–22

Ogata T, Kodama M, Komaru K, Sakamoto S, Sato S, Simidu U (1990) Production of paralytic shellfish toxins by bacteria isolated from toxic dinoflagellates. In: Granéli E, Sundström B, Edler L, Anderson DM (eds) Toxic marine phytoplankton. Proceedings of the 4th international conference on toxic marine phytoplankton. Elsevier, Amsterdam, pp 311–315

Riemann L, Steward GF, Fandino LB, Campbell L, Landry MR, Azam F (1999) Bacterial community composition during two consecutive NE monsoon periods in the Arabian sea studied by denaturing gradient gel electrophoresis (DGGE) of rRNA genes. Deep-Sea Res II 46:1791–1811

Riemann L, Steward GF, Azam F (2000) Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. Appl Environ Microbiol 66:578–587

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. 1–3, Ford N (chief ed) Cold Spring Harbor, New York

Schäfer H, Abbas B, Witte H, Muyzer G (2002) Genetic diversity of "satellite" bacteria present in cultures of marine diatoms. FEMS Microbiol Ecol 42:25–32

Seibold A, Wichels A, Schütt C (2001) Diversity of endocytic bacteria in the dinoflagellate *Noctiluca scintillans*. Aquat Microb Ecol 25:229–235

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Töbe K, Ferguson CMJ, Kelly MS, Gallacher S, Medlin LK (2001) Seasonal occurrence at a Scottish PSP monitoring site of purportedly toxic bacteria originally isolated from the toxic dinoflagellate genus *Alexandrium*. European J Phycol 36:243–256

Wichels A, Biel SS, Gelderblom HR, Brinkhoff T, Muyzer G, Schütt C (1998) Bacteriophage diversity in the North Sea. Appl Environ Microbiol 64:4128–4133

Zubkov M, Fuchs BM, Archer SD, Kiene RP, Amann R, Burkill PH (2001) Linking the composition of bacterioplankton to rapid turnover of dissolved dimethyl-sulphoniopropionate in an algal bloom in the North Sea. Environ Microbiol 3:295

Helgol Mar Res (2004) 58:140
DOI 10.1007/s10152-004-0183-5

ERRATUM

Antje Wichels · Christian Hummert · Malte Elbrächter · Bernd Luckas · Christian Schütt · Gunnar Gerdts

Bacterial diversity in toxic *Alexandrium tamarense* blooms off the Orkney Isles and the Firth of Forth

Published online: 6 May 2004

Helgoland Marine Research (2004) s10152-004-0174-6

The address of Malte Elbrächter should be as given above.

The Introduction should begin with the following sentence:

Populations of *Alexandrium tamarense* around the Orkney Islands produce potent neurotoxins, paralytic shellfish poisoning (PSP) toxins (Medlin et al. 1998).

The following reference should be included:

Medlin LK, Lange M, Wellbrock U, Donner G, Elbrächter M, Hummert C, Luckas B (1998) Sequence comparisons link toxic European isolates of *Alexandrium tamarense* from the orkney Islands to toxic North American stocks. Eur J Protistol 34:329–335

The online version of the original article can be found at http://dx.doi.org/10.1007/s10152-004-0174-6

A. Wichels (✉) · C. Schütt · G. Gerdts
Alfred-Wegener-Institut für Polar- und Meeresforschung,
Biologische Anstalt Helgoland,
27483 Helgoland, Germany
e-mail: awichels@awi-bremerhaven.de
Tel.: +49-4725-819245
Fax: +49-4725-819283

C. Hummert
Chemical and Biological Analyses, WEJ GmbH,
21107 Hamburg, Germany

M. Elbrächter
Deutsches Zentrum für Biodiversitätsforschung,
FI-Senckenberg, Wattenmeerstation Sylt,
Hafenstrasse 43, 25992 List/Sylt, Germany

B. Luckas
Department of Biology and Pharmacy,
Institute of Food Chemistry, Friedrich-Schiller-University Jena,
07743 Jena, Germany