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Analysis of bacterial diversity and metals in produced water, seawater and sediments from an offshore oil and gas production platform

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ABSTRACT

Produced water is one of the largest waste products routinely discharged into the ocean from offshore oil and gas platforms. This study analyzed bacterial communities and metals in the produced water, surrounding seawater, and sediment around the Thebaud platform. The bacterial community within the produced water was different from the seawater (SAB = 13.3), but the discharge had no detectable effect on the bacterial communities in the seawater (SAB > 97). In contrast, genomic analysis of sediments revealed that the bacterial community from 250 m was different (SAB = 70) from other locations further from the discharge, suggesting that the produced water had a detectable effect on the bacterial community in the sediment closest to the discharge. These near-field sediments contained elevated concentrations of manganese and iron that are associated with the produced water effluent. The results suggested that the discharge of produced water has influenced the bacterial community structure of sediments adjacent to the platform.

1. Introduction

Produced water, formation water residing within the geological formation and liquid chemicals introduced into the process stream to improve safety and enhance product recovery, is the largest waste product discharged into the surrounding seawater during oil and gas exploration and production operations. Produced water discharged from offshore oil and gas wells is usually anoxic and has a salinity and temperature that is different from that of the receiving water. It also usually contains elevated concentrations of metals, radionuclides, hydrocarbons, and ammonia compared to the receiving environment. In Canada, only the concentrations of hydrocarbons in the discharges are regulated. The discharges usually rely on dilution to decrease any environmental effects of anoxic conditions, temperature and salinity, or potential toxic effects of metals or other chemicals in the discharge. Although it is generally believed that the acute toxicity of produced water may be reduced by hydrocarbon removal and dilution of the plumes (Armsworthy et al., 2005), concerns remain over the potential chronic effects of discharges and the bioaccumulation of contaminants (Perez-Casanova et al., 2010). In order to understand the potential chronic effects of produced water and its zone of influence, the first step is to understand the transport and dilution of the chemicals in the produced water discharge.

Neff (2002) reviewed produced waters from around the world and found that several metals in produced water could be present at concentrations substantially more elevated (i.e. 1000 times or more) than in the surrounding seawater. The metals most frequently detected at elevated concentrations in produced water are barium, iron, manganese, and zinc. (cf. Neff, 2002). When discharged into well oxygenated, sulfate rich surface waters, dissolved iron and manganese can precipitate out as oxides (Azetsu-Scott et al., 2007). Iron and manganese oxides could therefore settle out of the water column and accumulate in sediments around the discharge (Neff, 2002; Lee et al., 2005). Depending on the current regime at the site of concern, these metal oxides could be dispersed over a large area, elevating their concentrations in seabed sediments. Our principal objectives were to examine the metal concentrations in the near-field sediments (<500 m from point of discharge) of an operational offshore platform and to characterize the bacterial community structure in the surrounding seawater and sediments with the goal to identify whether there was any evidence of effects from produced water discharge on the bacterial community.

2. Materials and methods

2.1. Sample collection

All 2002 sediment samples were collected during the Fisheries and Oceans Canada (DFO), cruise (2002-054) on board the

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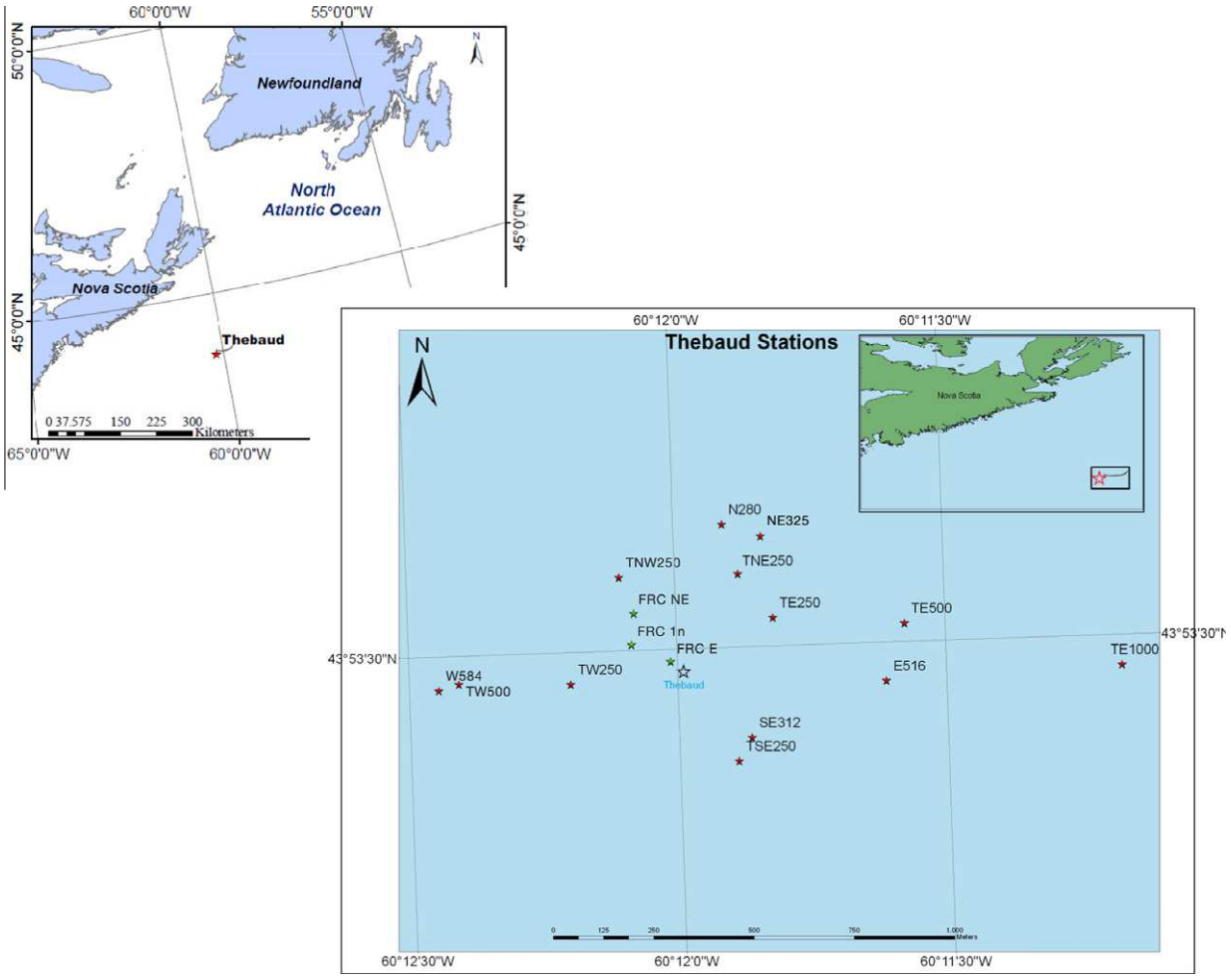

Fig. 1. Thebaud sampling locations. Sediment core sampling locations labeled with a red star and their names are represented by: e.g. TE250, T = Thebaud, E = East, 250 = 250 m away from platform. Seawater sampling locations labeled with a green star and their names are represented by only the direction from the platform. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

Canadian Coast Guard Ship (CCGS) Hudson from September 10–October 3, 2002 at stations radiating from the edge of the 250 m exclusion zone of the Thebaud platform (43° 53.5′N, 60° 12′W) on the Scotian Shelf off the coast of Nova Scotia, Canada (Fig. 1 and Table 1). The Thebaud platform is one of the largest gas and light oil production platforms on the eastern sea board of Canada. It is a gas-gathering center collecting gas from the Thebaud, the North Triumph and Venture wells before the gas is sent through a pipeline to a processing plant in Nova Scotia. All 2003 sediment samples were collected during the DFO Hudson Cruise (2003-059) from September 25–October 15, 2007. The 2007 samples were collected during the DFO Hudson Cruise (2007-036) from August 12–24, 2007. All sediment samples collected were at stations similar to 2002. The depth of the water column at the collection site was between 28 and 34 m with the produced water being discharged at 10 m below the surface. Seawater samples were collected at a depth of 10 m from three locations (FRC 1N, FRC NE, FRC E) next to the mouth of the produced water plume (Fig. 1 and Table 1) and from a reference station located 10 km west of the platform (R 10 km W) (Table 1).

2.2. Water sample collection

Seawater samples were collected using shipboard 10 L or 20 L Niskin bottles attached to a Conductivity, Temperature and Depth (CTD) Rosette to provide the temperature (°C), salinity (psu), and depth (meters) of the sample station. Samples of fresh produced water collected from the discharge process lines on the production platform were transferred at sea to our research vessel for simultaneous processing with our field samples. Samples were collected in two different types of containers: acid rinsed 10 L Nalgene® HDPE jerricans and solvent rinsed 4 L amber glass bottles. Containers used in the filtration were also rinsed three times with the sample water. After collection, samples were stored at 4 °C on the research ship until further processing. From the jerrican samples, an aliquot was removed for pH, salinity measurements and bacterial community analyses. Next, 4 L of seawater and 5 L of the produced water were immediately filtered through sterile 0.22 μm GSWP (Millipore) filters. Following filtration, all filters were transferred to sterile 50 mL Falcon tubes and stored at −20 °C until analyzed. For organic chemical analyses, four raw produced water samples were stored in amber glass bottles. Polycyclic aromatic hydrocarbons (PAH) and aliphatic hydrocarbons (alkanes) were determined using a modified version of EPA Method 8270. Alkylated and nonyl phenols (phenols) were analyzed using a modified version of EPA method 8041. All BTEX (Benzene, Toluene, Ethylbenzene, and Xylene) samples were stored at 4 °C and analyzed within 2 weeks of collection using modified EPA Method 8240 (purge and trap).

2.3. Sediment sample collection and analysis

Bottom sediment core samples were collected at a grid of stations radiating from the edge of the 250 m exclusion zone of the Thebaud platform (Fig. 1 and Table 1). The station numbers

Table 1
Bottom sediment and seawater sample station names, collection dates, locations, and water depth.

Station	Year	Latitude	Longitude	Water depth (m)
TE250-1	2002	43.8888	−60.1997	31
TE250-2	2002	43.8859	−60.1996	34
TE500-1	2002	43.8902	−60.1918	33
TE500-2	2002	43.8911	−60.1933	34
TE500-3	2002	43.8908	−60.1930	34
TNE250	2003	43.8933	−60.1981	31
TE250	2003	43.8914	−60.1959	31
TE500	2003	43.8914	−60.1925	34
TSE250	2003	43.8891	−60.1983	30
TW250	2003	43.8910	−60.2034	32
TW500	2003	43.8910	−60.2069	33
TNW250	2003	43.8933	−60.2018	31
N280	2007	43.8944	−60.1986	28
NE325	2007	43.8941	−60.1974	28
TE250-1	2007	43.8923	−60.1971	30
TE250-2	2007	43.8923	−60.1968	30
TE250-3	2007	43.8919	−60.1966	31
TE500-1	2007	43.8921	−60.1930	33
TE500-2	2007	43.8886	−60.1871	32
TE500-3	2007	43.8923	−60.1932	32
W584	2007	43.8909	−60.2075	29
TE1000	2007	43.8910	−60.1863	32
SE312	2007	43.8896	−60.1978	32
E516	2007	43.8908	−60.1936	34
FRC 1 N	2007	43.8918	−60.2015	N.D.
FRC NE	2007	43.8925	−60.2014	N.D.
FRC E	2007	43.8914	−60.2003	N.D.
R10kmW	2007	43.8890	−60.4312	N.D.

N.D. = Not Determined.

indicate both the direction and the distance from the platform (e.g. TE250, is 250 m east of the platform) (Table 1). Sampling consisted of deployment of a Bothner-type Slo-Corer (Law et al., 2008). The Slo-Corer applied a large driving force (~350 kg) onto a polycarbonate core barrel while hydraulically damping the rate of descent. This slow rate but large driving force prevented disturbance of the sediment water interface. Once full penetration was achieved, recovery began with the sealing of the core top by an o-ring and flange which prevented the loss of sediment as the barrel was withdrawn. As soon as the core barrel cleared the bottom, a gasketed spade plate covered the bottom of the core, thus sealing both the top and bottom of the core. The supernatant water column was retained with no free surface, thus insuring that the sediment water interface remained undisturbed even during rough recoveries. Once recovered and on deck the clear core liner was inspected for sample integrity. If any evidence of leakage from the bottom seal or disruption of the sediment–water interface was detected, the sample was discarded.

Collected cores were placed on an extruder and a series of 2 cm-thick sections were taken for the entire length of the core. The outer edges of the core were aseptically cut off and discarded, and only the center of the core was kept for chemical and biological analyses. For microbial analysis, 5–10 g of the core samples were transferred to sterile 50 mL Falcon tubes and stored at −20 °C until analyzed. For metal analysis, samples were stored at 4 °C in polyethylene cups with taped caps.

At the lab, samples were dried at <60 °C, sieved using a 1 mm stainless steel mesh and then sent out for trace metal analysis at the Research Productivity Council (RPC), Fredericton, N.B. At RPC, samples were digested in a mixture of concentrated nitric and hydrofluoric acids, taken to incipient dryness, and re-dissolved in a mixture of hydrochloric and nitric acids. After dilution to volume with deionized water, the samples were analyzed by Inductively Coupled Plasma-Emission Spectroscopy (ICP-ES) for higher concentration elements and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) for trace elements. To insure quality control, National Research Council (NRC) – marine sediment Certified Reference Materials, CRM's, (MESS-3, HISS-1) were included in the samples sent for analysis. RPC also has an extensive internal quality assurance protocol that included analysis of NIST CRMs 1646a and 2709.

2.4. Genomic DNA extraction

Total community DNA from seawater and produced water were extracted from the filter with an UltraClean® Water DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA) following the manufacturer's protocol. Total community DNA from 0–2 cm sediment samples was extracted from 2 g of sediment with an UltraClean® Soil DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA) following the manufacturer's protocol. DNA concentration was estimated by electrophoresis on a 0.7% agarose gel using 5 μL of purified material and the Lambda *Hind*III DNA ladder (Amersham Biosciences, Piscataway, NJ) standard.

2.5. Molecular analysis

PCR amplification of the 16S rRNA gene, Denaturing Gradient Gel Electrophoresis (DGGE) analysis and phylogenetic analysis were performed as previously described (Yeung et al., 2010). PCR amplification of the 16S rRNA gene was carried out by using the bacteria-specific forward primer U341F (5′-CCTACGGGAGGCAGCAG-3′) (Muyzer et al., 1993) and the reverse primer U758R (5′-CTACCAGGGTATCTAATCC-3′) (Fortin et al., 1998). This primer set, complementary to conserved regions of the 16S rRNA gene, were used to amplify a 418-bp fragment corresponding to positions 341 to 758 in the *Escherichia coli* sequence and covered the variable regions V3 and V4. The bacteria-forward primer used for DGGE incorporated a GC clamp (5′-GGCGGGGCGGGGGCACGGGGGGCGCGGCGGGCG GGGCGGGGG-3′) at the 5′ end. This GC-clamp stabilizes the melting behavior of the amplified fragments (Sheffield et al., 1989). Each 50 uL PCR mixture contained ~1 ng/μL of the template DNA, 25 pmol of each oligonucleotide primer, 200 μM of each dNTP, 1 mM $MgCl_2$, 2.5 units of Taq polymerase (Amersham Biosciences, Piscataway, NJ, USA) and 1x Taq polymerase buffer (10 mM Tris–HCl pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$). Amplification was performed using the protocol from Yeung et al. (2010). PCR products were loaded onto a 1% agarose gel with SYBR Safe (Molecular Probes, Eugene, OR, USA), using a 100-bp ladder (MBI Fermentas, Amherst, NY, USA) to determine the presence, size and quantity of the PCR products.

The 16S rRNA gene PCR products from four PCR reactions were combined for each sample and concentrated by ethanol precipitation for DGGE analysis. About 650 ng of 16S rRNA gene PCR product from each sample was applied to a lane, and was analyzed on 8% polyacryalmide gels containing gradients of 35–65% denaturant (7 M urea and 40% deionized formamide were considered to be 100% denaturant) as described in Yeung et al. (2010). Briefly, DGGE was performed with a DCode Universal Mutation Detection System (Bio-Rad, Mississauga, ON, Canada) at a constant voltage of 80 V for 16 h at 60 °C in 1x TAE running buffer. The gels were stained with SYBR Gold Nucleic Acid Gel Stain (Molecular Probes, Eugene, OR, USA) and imaged with the FluoroImager System Model 595 (Molecular Dynamics, Sunnyvale, CA, USA). The gel images were analyzed with GelCompar II v4.6 (Applied Maths, Sint-Martens-Latem, Belgium) to generate dendrogram profiles. The genotypes were visually detected based on presence or absence of bands in the different lanes. A band was defined as "detected" if the ratio of its peak height to the total peak height in the profile was >1%. After conversion and normalization of gels, the degrees of similarity of DNA pattern

profiles were computed using the Dice similarity coefficient (Dice, 1945), and dendrogram patterns were clustered by the unweighted pair group method using arithmetic average (UPGMA) groupings with a similarity coefficient (S_{AB}) matrix.

Individual bands from the DGGE gels were excised and eluted with 25 μL of dH_2O for 48 h at 4 °C before being re-amplified with the same set of primers without the GC-clamp. 1 μL of DNA was re-amplified as follows: an initial denaturation of 5 min at 96 °C, followed by 30 cycles of 94 °C for 1 min, 65 °C for 30 s, and 72 °C for 1 min. PCR products for sequencing were purified using Illustra GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare, Piscataway, NJ). Sequencing was performed at the Université Laval Plate-forme d'analyses biomoléculaires using a model ABI Prism 3130XL (Applied Biosystems, Foster City, CA) with their respective primers. Raw sequence data were assembled in BioEdit v7.0.5.3 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html, Hall 1999). The sequences were manually aligned by comparing forward and reverse sequences. The occurrence of chimeric sequences was determined manually with Bellerophon (http://foo.maths.uq.edu.au/~huber/bellerophon.pl, Huber et al. 2004). Close relatives of the final selection of different sequences (phylotypes) were tentatively identified by NCBI BLASTN search (http://www.ncbi.nlm.nih.gov/blast/). Sequences were aligned by the MacVector 9.0 software package (Accelrys, Cary, NC) with both closely-related representatives from NCBI BLASTN and as well as novel complete and partial sequences obtained from GenBank. Additional manual alignment was done if necessary. Phylogenetic relationships were constructed with evolutionary distances (Jukes–Cantor distances) and the neighbor-joining method using the MacVector software package. The bootstrap analyses for the phylogenetic trees were calculated by running 1000 replicates of the neighbor-joining data.

2.6. Nucleotide sequence accession numbers

The 16S rRNA gene sequences obtained in this study were deposited in the GenBank database under accession numbers HQ852399 to HQ852438.

3. Results

3.1. Physicochemical analyses of the produced water and surrounding seawater

The pH of the produced water was 5 and the salinity was 12.3 ppt. The produced water contained a mixture of petroleum hydrocarbon constituents: 50 ± 18 mg/L BTEX, 180 ± 30 mg/L phenols, 0.13 ± 0.18 mg/L total alkanes, and 2.15 ± 0.28 mg/L PAHs. The high standard deviation, based on the analysis of four samples collected at different times, reflects the temporal variability in the concentrations of produced water constituents. In contrast, the surrounding seawater was 13.6 °C with a salinity of 32 ppt. Petroleum hydrocarbon concentrations in the sediments (~250 m from the point of discharge) were below detection limits (data not shown).

3.2. Produced water and bottom sediment trace metal analysis

The produced water discharged from the Thebaud facility contained a variety of metals at elevated concentrations, including aluminum, barium, calcium, iron, lithium, magnesium, manganese, potassium, sodium, and strontium (Table 2). Metals like calcium, magnesium, potassium, sodium, and strontium can be elevated in seawater (Table 2) and contribute to overall produced water concentrations when injected into the formation to maintain well pressure. As a result, focus was given to metals that were distinctly associated with the formation water (i.e. barium (Ba), iron (Fe), and manganese (Mn)).

Table 2
Trace metal concentration in Thebaud produced water and seawater.

	Concentration (μg/L)	
	Produced water	Seawater
Aluminum	25	1
Antimony	<2	0
Arsenic	<50	2.6
Barium	58,000	21
Beryllium	<0.1	0.0006
Bismuth	<0.5	0.02
Boron	2200	4450
Cadmium	<0.02	0.11
Calcium	1,220,000	411,000
Chromium	<10	0.2
Cobalt	<10	0.39
Copper	<10	0.9
Iron	10,300	3.4
Lanthanum	1	0.0029
Lead	1.2	0.03
Lithium	860	170
Magnesium	85,200	1,290,000
Manganese	1270	0.4
Molybdenum	0.94	10
Nickel	<20	6.6
Phosphorus	50	88
Potassium	45,800	392,000
Rubidium	140	120
Selenium	<50	0.9
Silicon	1270	2900
Silver	<0.2	0.28
Sodium	2,910,000	10,800,000
Strontium	102,000	8100
Sulfur	330	904,000
Tellurium	<2	N.D
Thallium	5	N.D.
Thorium	<0.2	0.0004
Tin	<0.5	0.81
Titanium	<1	1
Uranium	0.006	3.3
Vanadium	<5	1.9
Zinc	27	5

N.D. = Not detectable; seawater values from Turekian, 1968.

Geochemical normalization of specific metals within bottom sediments to 'conservative' elements, such as lithium (Li) and aluminum (Al), has been used with varying degrees of success to differentiate natural accumulation from anthropogenic inputs, and to reduce the trace metal variability caused by grain size as well as by mineralogy (Loring, 1990; Hirst, 1962a,b). While Loring (1990) determined that Li is a good standard for normalization of samples from eastern Canadian estuarine and coastal sediments, Muschenheim et al. (2010) reported that normalization of metal concentrations vs. Li failed to produce a good correlation in samples from the sediments of Sable Island Bank because of the detection limitations in the Li data. They suggested the use of Al as a good alternative since it provided geochemical grain-size normalization for the metals in the Scotian Shelf sediments and does not suffer from the same detection limit problems that were observed in the Li normalization (Muschenheim et al., 2010). In consideration of this previous work, Al was used to normalize all the other metal data from the sediment in this analysis. Average background concentrations and 5% confidence intervals, based on normalization to Al, were determined from the sample analysis and regression of over 500 bottom sediment samples collected from the Scotian Shelf (Yeats, Unpublished data).

The results found elevated normalized concentrations of Ba, Fe, and Mn in the sediments closest to the platform (Figs. 2–4). Barium

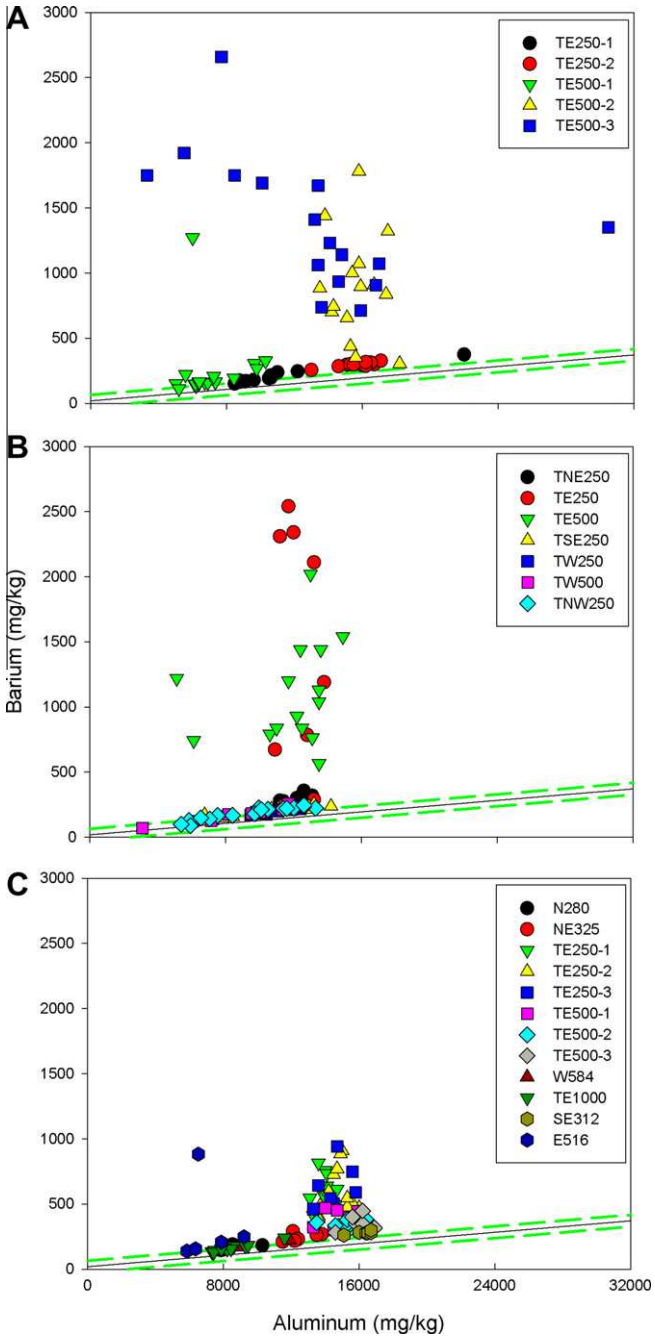

Fig. 2. Plots of trace metal analysis from sediment cores taken in the vicinity of the Thebaud platform. Trace metal data has been plotted against aluminum to remove the effects of grain size. A. Plot of barium concentration (mg/kg) vs. aluminum from samples taken in 2002. Core location names are represented by: (e.g. TE250, T = Thebaud, E = East, 250 = 250 m away from platform, −1, −2 represent core 1 and core 2 from the same location. B. Barium concentration vs. aluminum from cores collected in 2003. C. Cores collected in 2007. Sediment cores were sectioned into 2 cm intervals down core and samples from each core represent a 2 cm homogenized sample. Solid black line and green dashed lines represent average background concentration of barium based on aluminum concentration and the 5% confidence intervals, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

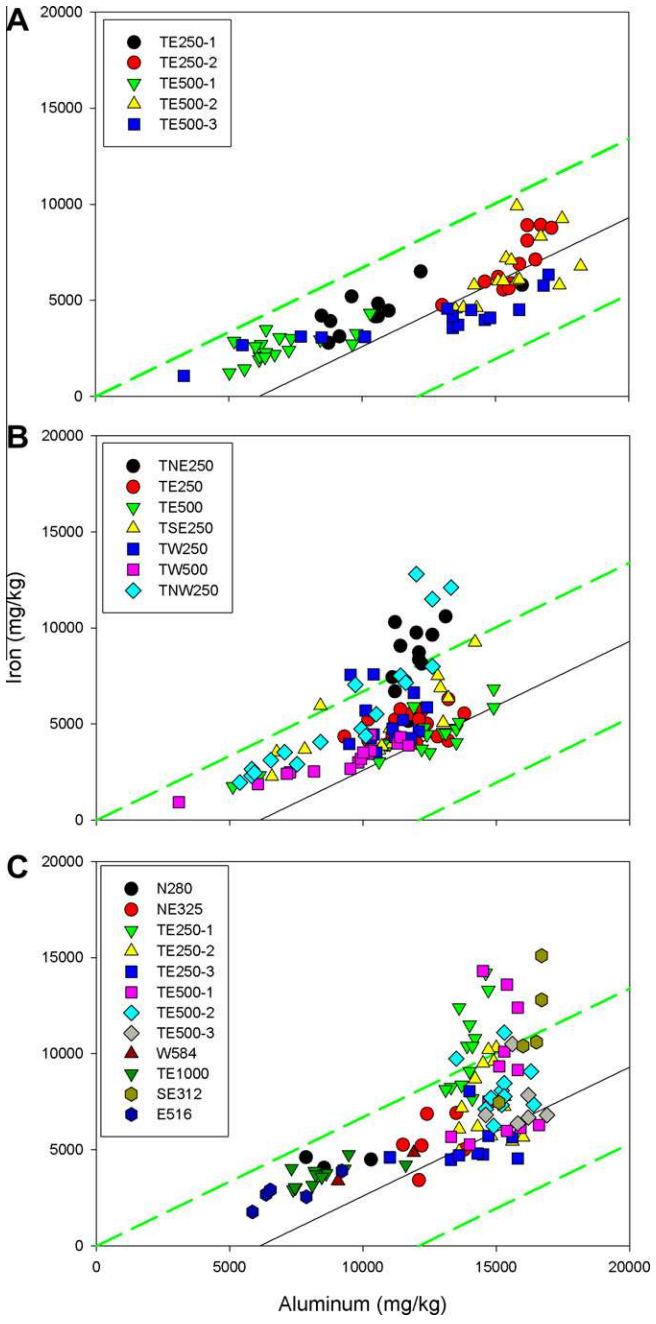

Fig. 3. Plots of trace metal analysis from sediment cores taken in the vicinity of the Thebaud platform. Trace metal data has been plotted against aluminum to remove the effects of grain size. A. Plot of iron concentration (mg/kg) vs. aluminum from samples taken in 2002. Core location names are represented by: (e.g. TE250, T = Thebaud, E = East, 250 = 250 m away from platform, −1, −2 represent core 1 and core 2 from the same location. B. Iron concentration vs. aluminum from cores collected in 2003. C. Cores collected in 2007. Sediment cores were sectioned into 2 cm intervals down core and samples from each core represent a 2 cm homogenized sample. Solid black line and green dashed lines represent average background concentration of barium based on aluminum concentration and the 5% confidence intervals, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

concentrations showed some temporal differences with concentrations varying from background values up to 2500 mg/kg (Fig. 2). The difference in concentration generally corresponded to the distance from the platform with the highest values associated with the sample stations at 250 and 500 m to the east of the platform (Fig. 2). Iron concentrations exhibited a noticeably temporal increase from 2002 to 2007 at the sample location occupied at 250 and 500 m to the east of the platform with the highest values approaching 15000 mg/kg from the samples collected in 2007 with near baseline values from samples in 2002 (Fig. 3). Samples collected at stations in 2003 (i.e. TNW250, TNE250, TSE250, TW250)

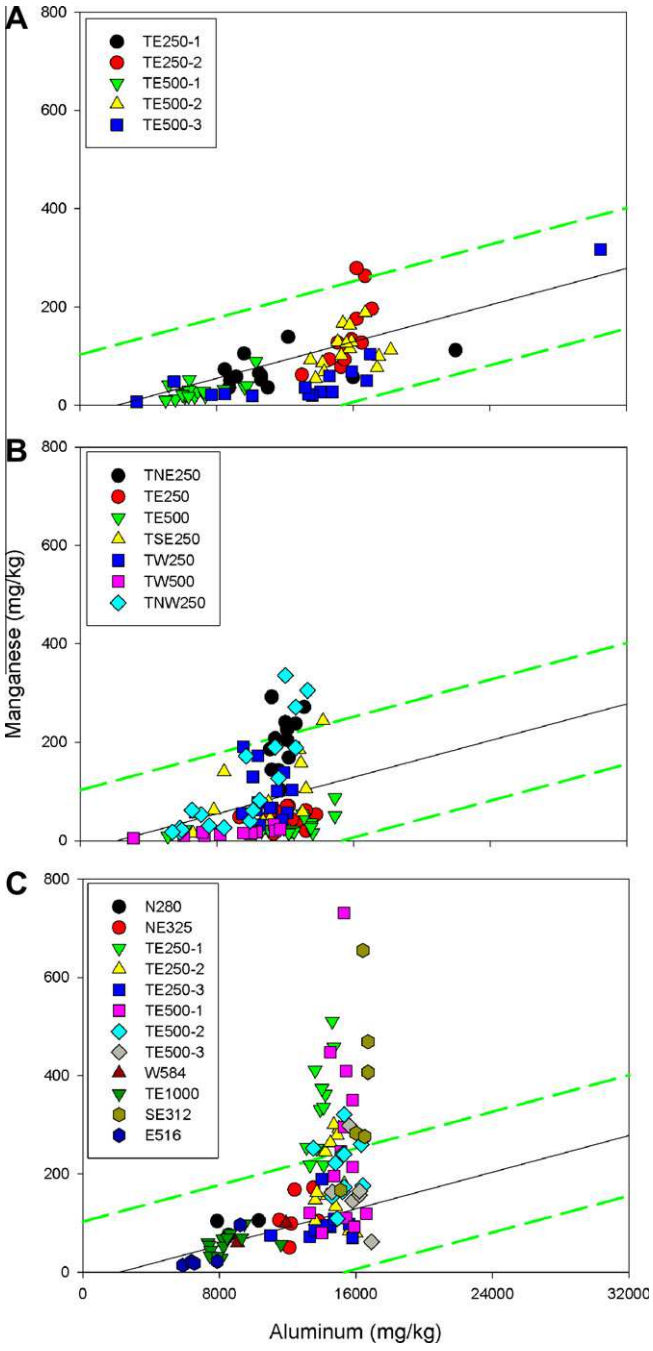

Fig. 4. Plots of trace metal analysis from sediment cores taken in the vicinity of the Thebaud platform. Trace metal data has been plotted against aluminum to remove the effects of grain size. A. Plot of manganese concentration (mg/kg) vs. aluminum from samples taken in 2002. Core location names are represented by: (e.g. TE250, T = Thebaud, E = East, 250 = 250 m away from platform, −1, −2 represent core 1 and core 2 from the same location. B. Manganese concentration vs. aluminum from cores collected in 2003. C. Cores collected in 2007. Sediment cores were sectioned into 2 cm intervals down core and samples from each core represent a 2 cm homogenized sample. Solid black line and green dashed lines represent average background concentration of barium based on aluminum concentration and the 5% confidence intervals, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

and 2007 (i.e. SE312) also showed elevated values for iron when compared to background values. Results suggest that the increase in iron concentration might be correlated to the produced water discharge. Similar to the iron data, the highest manganese concentrations also demonstrated similar temporal and spatial patterns with the highest values from samples closest to the discharge and from the most recent year (Fig. 4). This combination of the temporal and spatial trends from barium, iron, and manganese suggest that the accumulation of these metals in the sediment may be related to the cumulative input of the produced water with time. The well has been active since December, 1999.

3.3. DGGE analyses

DGGE analysis revealed that the bacterial community structure from the produced water was very different from the surrounding seawater (S_{AB} = 13.3, Fig. 5), even though the major component of the produced water was the injected surrounding seawater. The seawater DGGE results showed that the bacterial community structure was virtually identical ($S_{AB} \geqslant 97.1$, Fig. 5) in all the samples from the mouth of the produced water plume to 10 km away from the platform, suggesting that the bacterial community structure was spatially very stable in the surrounding seawater.

Similarly, the produced water/sediment DGGE analysis also revealed that the bacterial community structure from the produced water was very different from the sediment (S_{AB} = 26, Fig. 8). The sediment DGGE results revealed that there was a high similarity in the bacterial community structure in the sediments which clustered into two major groups: one from samples from the 250 m locations and the other from samples beyond 250 m (S_{AB} = 70.2, Fig. 8). This suggests that the bacterial community in the sediment from the 250 m locations is detectably different from the bacterial community located further away from the platform.

3.4. Phylogenetic analysis of produced water

The other purpose of the bacterial 16S rRNA gene DGGE analysis was intended to identify the dominant bacterial groups in the produced water, seawater and sediment, so all the major bands were excised and re-amplified for sequence analysis. From the produced water DGGE, a total of four DGGE bands were excised and sequenced (Figs. 5 and 8). All of the sequences showed at least a 97% match to GenBank sequences related to *Acinetobacter* sp. (1 sequence) and *Geobacillus* sp. (3 sequences) from the *Gamma-proteobacteria* and *Firmicutes*, respectively (Fig. 6).

3.5. Phylogentic analysis of seawater and sediments

Both seawater and sediment DGGE displayed a much higher bacterial diversity (i.e. higher number of bands) than the produced water (Figs. 5 and 8). From the seawater DGGE, a total of 15 bands were excised and sequenced (Fig. 5). Most of the sequences (except TBSW-3 and -10) showed at least a 97% match to sequences in GenBank, and most of the closest matches were related to *Proteobacteria* and *Bacteroidetes* from the marine environment (Fig. 7). Within the *Proteobacteria*, the phylum can be divided into *Alpha-* and *Gamma-proteobacteria* with 4 sequences and 1 sequence, respectively (Fig. 7). The highest diversity was in the *Bacteroidetes* group with 10 sequences (Fig. 7).

The highest bacterial diversity was found in the sediment with a total of 21 DGGE bands excised and sequenced (Fig. 8). Most of the sequences showed at least a 97% match to sequences in GenBank from marine sediments or seawater from the *Actinobacteria*, *Proteobacteria* and *Bacteroidetes* groups. Similar to the seawater, most of the sequences (14 out of 21) belonged to *Bacteroidetes*. Only 2 sequences were related to the *Actinobacteria* and 5 sequences belonged to the *Proteobacteria* including the *Delta-*, *Gamma-*, and *Epsilon-proteobacteria* subgroups. As previously mentioned, there was some uniqueness in the bacterial community in the sediment samples from the 250 m locations, in which the only *Epsilon-proteobacteria* were identified (Fig. 9).

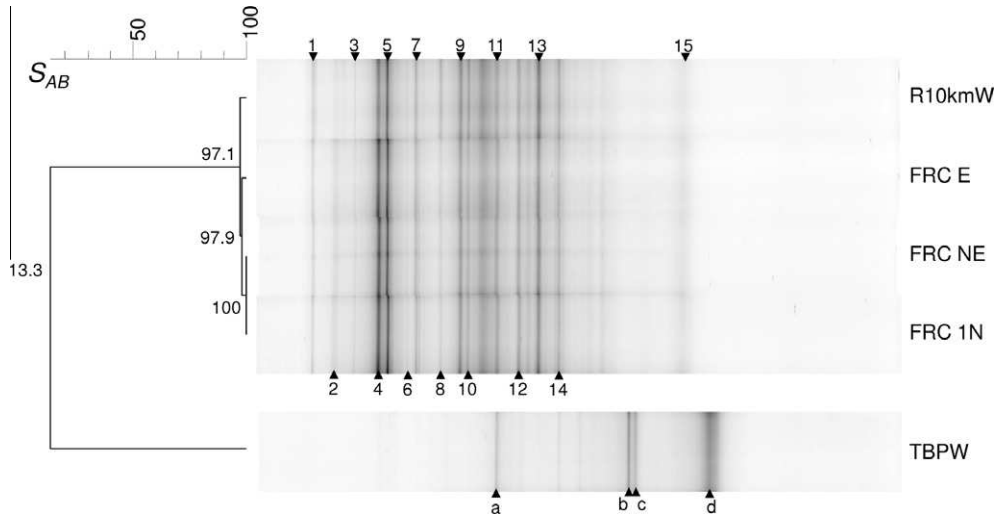

Fig. 5. DGGE fingerprint cluster analysis from surrounding seawater samples listed in Table 1 and a Thebaud produced water sample (TBPW). Seawater bands were labeled from 1–15 and produced water bands were labeled from a–d.

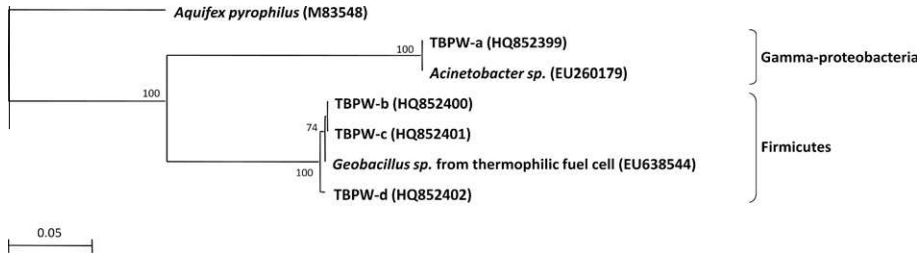

Fig. 6. Phylogenetic relationship of the four bacterial 16S rRNA gene sequences obtained from Thebaud produced water DGGE. The bands were labeled with TBPW – and the corresponding letter from Fig. 5. The tree was inferred by neighbor-joining analysis of the sequence from each clone. *Aquifex pyrophilus* was used as the outgroup. Numbers on the nodes are the bootstrap values based on 1000 replicates. The scale bar indicates the estimated number of base changes per nucleotide sequence position.

4. Discussion

4.1. Bottom sediment trace metal monitoring

Trace metals, such as barium, iron and manganese, had noticeably higher concentrations in the produced water (Table 2) than commonly found in seawater (Table 2) and the reference sediment (data not shown). Therefore, monitoring the concentrations of these metals in the surrounding environment could potentially be used as a method to define the discharge and dilution pattern of the produced water constituents.

The highest concentrations of barium, iron and manganese were found mainly in samples collected closest to the production platform in the most recent sampling year (Figs. 2–4), suggesting that the higher concentrations of these metals might be linked to increases in annual discharge rates and/or the cumulative discharge of the produced water with time. Evidently, the highest concentrations of trace metals associated with raw produced water were found in the sediments mainly to the east of the platform which is in the mean current drift direction (Hannah et al., 2001). Yeats et al. (in press) argued that the higher concentrations of barium could be the result of sedimentary Ba that originated from drilling muds, however, they also pointed out that drilling mud would not necessarily explain the above background observations of Fe and Mn concentrations that were found in the sediments closest to the production platform. This temporal trend of increased iron and manganese suggests that the accumulation was most likely related to the input from the produced water discharge. The metal results also revealed the potential transport and dilution pattern of the produced water, suggesting that constituents of produced water would travel downward in the water column, settle onto and bury into the sediment near the platform. This result further suggested that any potential biological impact from the effluent of produced water would most likely be at the sediment level close to the production platform.

4.2. Produced water bacterial diversity

Compared to produced waters from other petroleum production regions like Hibernia (Yeung et al., in press), Terra Nova (Yeung et al., Unpublished results), and the North Sea (Kaster et al., 2009), the bacterial community structure in the produced water recovered from the discharge lines of the Thebaud platform was relatively simple with only four major bands detected by DGGE. The sequences from these bands were closely related to *Acinetobacter* spp. and *Geobacillus* spp. These genera were not found in other produced waters from nearby regions (Yeung et al., Unpublished results; Kaster et al., 2009; Dahle et al., 2008), suggesting that the bacteria from the Thebaud produced water were unique to this produced water.

Most *Acinetobacter* isolates are classified as mesophilic, strictly aerobic and non-fermentative bacteria. Only a few *Acinetobacter*-like sequences or isolates have been identified from petroleum environments, such as produced water from high-temperature reservoirs in oilfields in China (Li et al., 2007; Nazina et al., 2005), suggesting that finding *Acinetobacter* spp. in the produced water in this region was not impossible, but uncommon for Atlantic oilfields. One of the explanations for finding *Acinetobacter* in the Thebaud

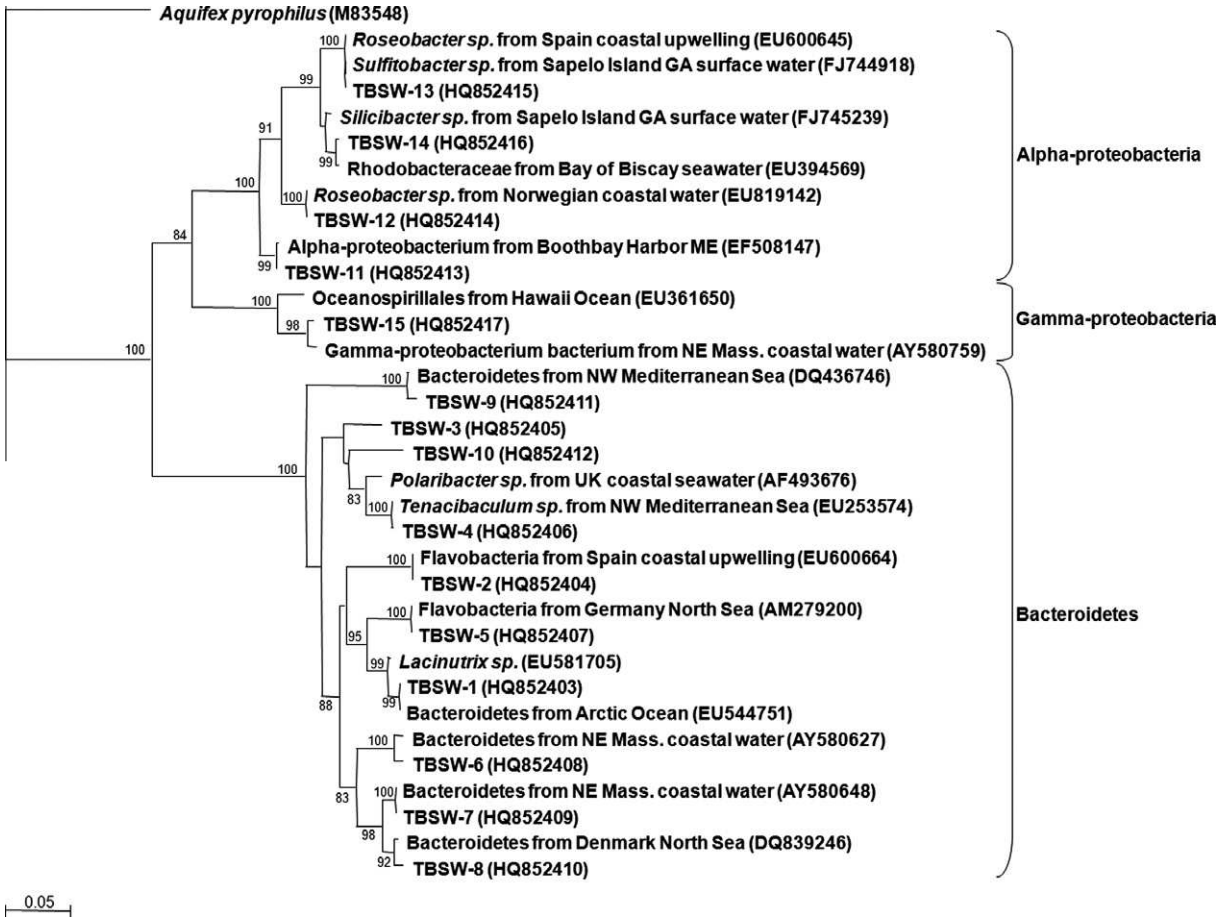

Fig. 7. Phylogenetic relationship of the 15 bacterial 16S rRNA gene sequences obtained from Thebaud seawater DGGE. The bands were labeled with TBSW – and the corresponding number from Fig. 5. The tree was inferred by neighbor-joining analysis of the sequence from each clone. *Aquifex pyrophilus* was used as the outgroup. Numbers on the nodes are the bootstrap values based on 1000 replicates. The scale bar indicates the estimated number of base changes per nucleotide sequence position.

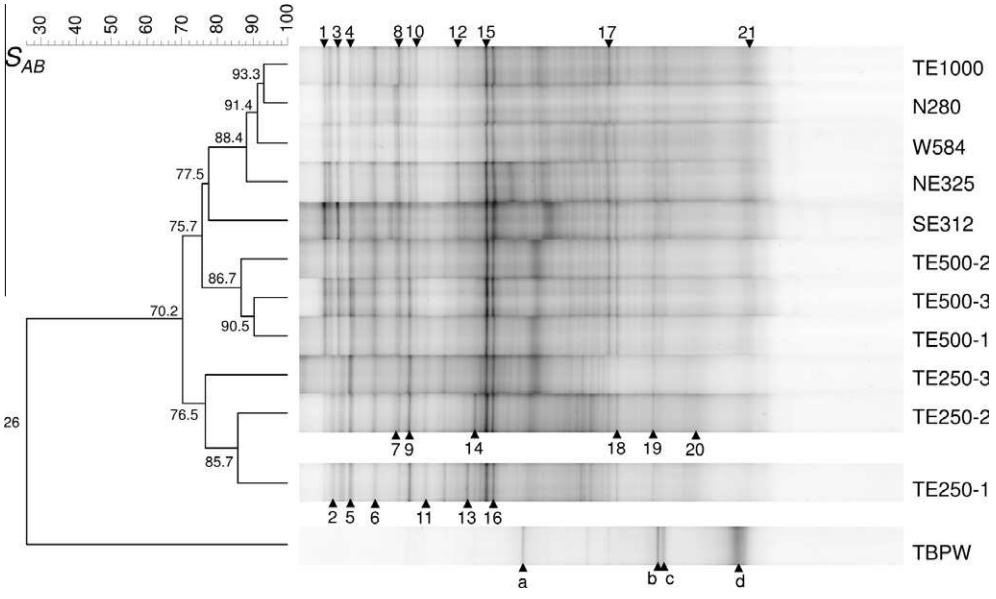

Fig. 8. DGGE fingerprint cluster analysis from surrounding sediment samples listed in Table 1 and a Thebaud produced water sample. Sediment bands were labeled from 1–21 and produced water bands were labeled from a–d.

produced water could be their relationship with hydrocarbons. Isolates of *Acinetobacter* are known to be able to use hydrocarbons as sole carbon and energy sources (Le Petit et al., 1975), and the *Acinetobacter* sp. detected in the Thebaud produced water may

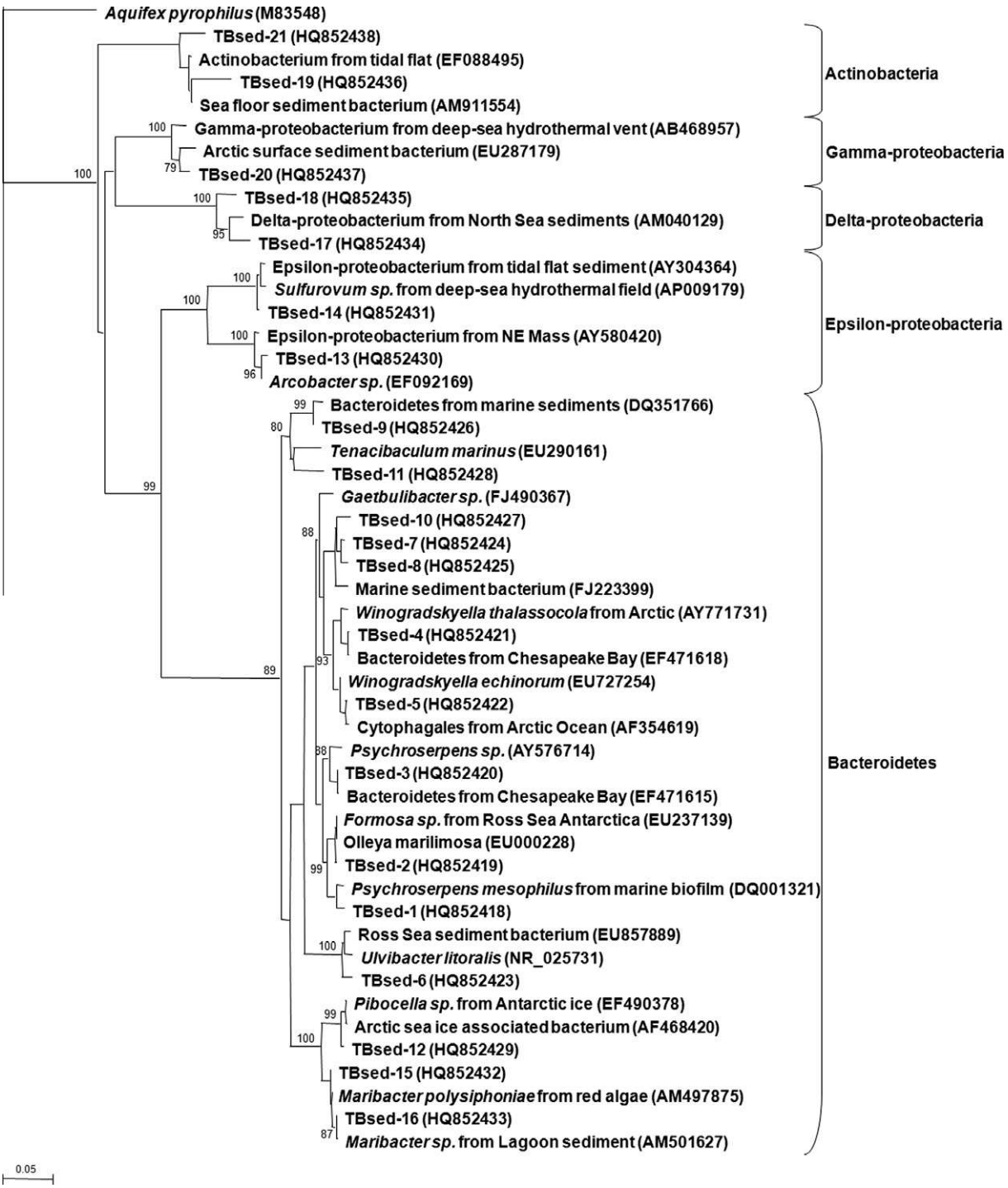

Fig. 9. Phylogenetic relationship of the 21 bacterial 16S rRNA gene sequences obtained from Thebaud sediment DGGE. The bands were labeled with TBsed – and the corresponding number from Fig. 8. The tree was inferred by neighbor-joining analysis of the sequence from each clone. *Aquifex pyrophilus* was used as the outgroup. Numbers on the nodes are the bootstrap values based on 1000 replicates. The scale bar indicates the estimated number of base changes per nucleotide sequence position.

originate from the cooler portions of the oil production environment or be present in treating agents (e.g. emulsion breakers, corrosion inhibitors, etc.) that are pumped down the well.

In the geothermally heated oil reservoirs, the petroleum hydrocarbons could also create a unique ecological niche for thermophilic, aerobic or facultative anaerobic, hydrocarbon-degrading bacteria (Nazina et al., 2001, 2005). *Geobacillus* spp. are known to be physiologically versatile and function in both thermophilic and mesophilic environments as aerobes or facultative anaerobes. Nazina et al. (2001) isolated five strains of bacteria belonging to *Geobacillus* from the formation waters of oilfields in Russia, Kazakhstan and China. These strains were all able to degrade a wide range of hydrocarbons, lower alcohols, and organic acids, suggesting that, similar to *Acinetobacter* spp., the *Geobacillus* sp. from Thebaud produced water might also be utilizing the hydrocarbons. However, neither *Acinetobacter* or *Geobacillus* are strictly anaerobic bacteria that are more commonly found in produced waters (Magot et al., 2000; Birkeland, 2004), suggesting that they might

not be indigenous to the anoxic petroleum formation. The presence of these genera in Thebaud produced water suggests that the produced water and the petroleum production system are providing a suitable environment for their survival and/or growth.

4.3. Seawater and sediment bacterial DGGE analysis

Yeung et al. (in press) and Yeung et al. (Unpublished results) found that there was a stable bacterial community in the surrounding seawater around the Hibernia and Terra Nova production platforms and suggested that this could be an indication that there were no detectable changes on the bacterial community from the produced water. It was also suggested that any effects from the produced water might be restricted to the region immediately adjacent to the platform or at the sediment level. The Thebaud seawater DGGE results revealed that the bacterial community structure in the seawater was also very uniform in the region immediately adjacent to the platform and suggested that the discharge of produced water did not have a detectable impact on the seawater bacterial community (Fig. 5). The sediment DGGE results showed that there were some differences in the banding patterns in the sediments from samples closest to the platform (250 m) (Fig. 8), possibly related to the proximity to the produced water discharge. The results revealed that sequences from the unique DGGE bands belonged to members from the *Epsilon-proteobacteria* that are closely related to *Sulfurovum* and *Arcobacter* (Fig. 9). No other *Epsilon-proteobacteria* were found in the seawater or other sediment samples (Figs. 7 and 9), suggesting that these *Epsilon-proteobacteria* were unique to these samples and might be related to the influence from the produced water discharge.

4.4. Epsilon-proteobacteria subgroup

Sulfurovum spp. have been isolated since 2004 (Inagaki et al., 2004), but very little is known about these bacteria. *Sulfurovum* is a sulfur-oxidizing and/or symbiotic bacterium and is only isolated from deep-sea hydrothermal vent environments (Tokuda et al., 2008; Inagaki et al., 2004), suggesting that they might originate from environments associated with thermophilic formations.

Members of the genus *Arcobacter* are better known and are usually classified as nitrate-reducing and sulfide-oxidizing bacteria. Most of the *Arcobacter* isolates were identified as potential pathogens associated with humans and livestock (Vandamme et al., 1992), so most of the phylogenetic and physiological studies conducted on this genus were pathogen-related studies. From these studies, *Arcobacter* spp. were generally found to grow under aerobic and microaerophilic conditions requiring 3–10% oxygen and they could grow over a wide temperature range (15–42 °C) (Snelling et al., 2006). Recently, *Arcobacter* spp. have been found in natural marine and lake environments, such as a Hawaiian hypersaline lagoon (Donachie et al., 2005), Black Sea sediment (Thamdrup et al., 2000), the North Sea (Eilers et al., 2000) and Solar Lake (Teske et al., 1996). Donachie et al. (2005) isolated the first obligate halophilic *Arcobacter* (later named *Arcobacter halophilus*) from a hypersaline lagoon in the Hawaiian Islands. *Arcobacter* spp. have also been isolated from oil fields in Saskatchewan, Canada (Gevertz et al., 2000), identified in a high-temperature offshore petroleum reservoir in the North Sea (Kaster et al., 2009) and detected in the Hibernia produced water discharge (Yeung et al., Unpublished results). Kaster et al. (2009) also demonstrated that sequences similar to *Arcobacter*, a sulfur-compound oxidizing *Epsilon-proteobacterium*, were enriched in acetogenic and fermentative media at 55 °C as well as fermentative and sulfate reducing media at 70 °C. *Arcobacter* spp. were also found in lower temperature environments from a petroleum degrading wetland soil from the Shengli Oil Field on the Yellow River delta (Han et al., 2009) and from the produced water in a crude oil gathering and transferring system (Liu et al., 2009). Liu et al. (2009) demonstrated that the *Arcobacter* were only found in the produced water but not in the crude oil, which suggests that *Arcobacter* might not originate from the petroleum formation but may become enriched by the hydrocarbons in the produced water. Therefore, finding *Epsilon-proteobacteria* in the sediment close to the platform, their unique physiological characteristics and their relationship to other oil fields suggested that these bacteria could be related to the produced water discharge.

4.5. Relationship between Fe, Mn and Arcobacter

Arcobacter spp. have been identified as iron- and/or manganese-reducing bacteria and may be able to utilize the elevated concentrations of iron and manganese that were found in the sediments around Thebaud. Otth et al. (2005) found *Arcobacter butzleri* from various sources were all resistant to relatively high concentrations of Mo, Mn, Ni, Co, Pb, and Fe. Thamdrup et al. (2000) found that *Arcobacter* sequences were the only sequences identified from the 16S rRNA gene clone libraries from manganese-reducing sediments from the Black Sea. Most dissimilatory Mn reducers are also known as dissimilatory Fe-reducing bacteria, using Fe (III) as an electron acceptor (Bowman et al., 1997; Ehrlich, 1993; Laverman et al., 1995). This suggests that *Arcobacter* spp. could play an important role in both iron and manganese reduction in the Thebaud sediments.

5. Conclusions

The results of this study provided the first bacterial community structure characterization of produced water from an offshore platform on the Scotian Shelf of Atlantic Canada, the surrounding seawater, and the surrounding sediment near the Sable Island Bank. The results revealed that the bacterial community profiles in various seawater samples from around the Thebaud offshore oil and gas production platform (within 250 m) were very similar with $S_{AB} > 97$, suggesting that the produced water did not have a detectable effect on the microbial populations in the surrounding water. However, sediment analysis revealed that the bacterial community profiles from 250 m samples were different from other locations further away from the production platform with $S_{AB} = 70$. *Arcobacter* was found as a major band on the DGGE only in the 250 m sediment samples, suggesting that the produced water might have some detectable effect on the bacterial populations in sediment adjacent to the discharge. Similarly, sediment metal analysis revealed that higher concentrations of manganese and iron were found in the sediment samples near the platform. The manganese and iron concentrations were also elevated in the Thebaud produced water. Both microbial and metal analyses suggested that the influence from produced water is restricted to the region adjacent to the platform at the sediment level. The flow rate and volume of Thebaud produced water is very small compared the other oil and gas production platforms (i.e. Hibernia and Terra Nova) in the region. If the discharge of Thebaud produced water has influenced the microbial community structure and the metal composition in sediments adjacent to the platform, similar effects could be expected in other production platforms with higher flow rates and volumes.

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