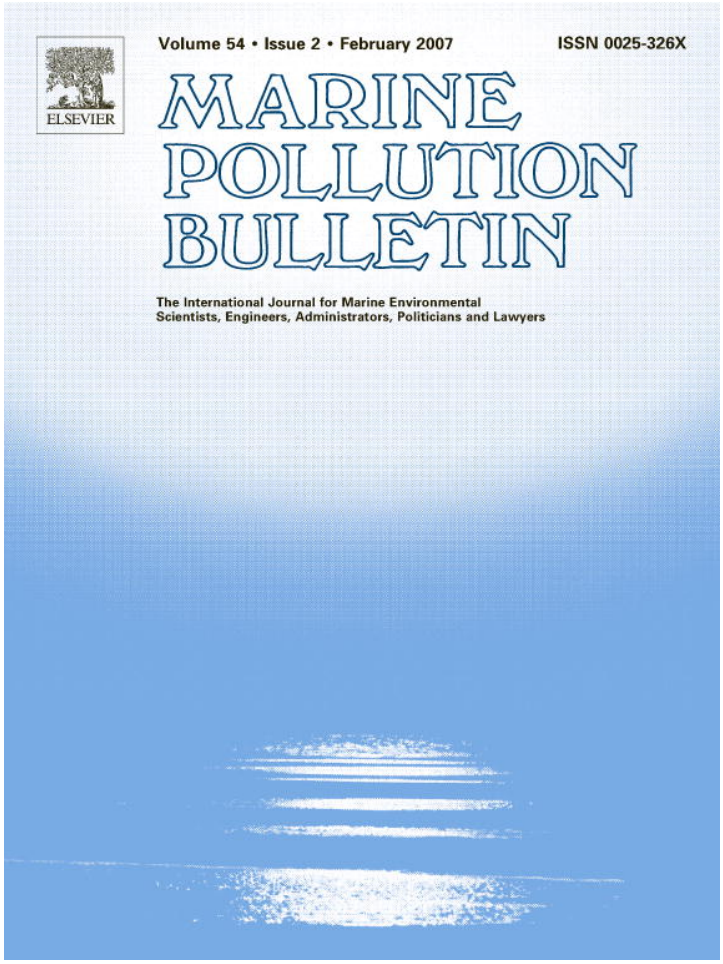

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Microbial community of cyanobacteria mats in the intertidal zone of oil-polluted coast of Saudi Arabia

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Abstract

Cyanobacterial mats are found at various locations along the coast of the Eastern Province of Saudi Arabia. Those mats were affected by severe oil pollution following 1991 oil spill. In this study, samples from Abu Ali Island were collected at three selected sampling sites across the intertidal zone (Lower, Middle, and Upper) in order to understand the effect of extreme environmental conditions of high salinity, temperature and desiccation on distribution of cyanobacteria along the oil polluted intertidal zone. Our investigation of composition of cyanobacteria and diatoms was carried out using light microscopy, and Denaturant Gradient Gel Electrophoresis (DGGE) technique. Light microscopy identification revealed dominant cyanobacteria to be affiliated with genera *Phormidium*, *Microcoleus*, and *Schizothrix*, and to a lesser extent with *Oscillatoria*, *Halothece*, and various diatom species. The analysis of DGGE of PCR-amplified 16S rRNA fragments showed that the diversity of cyanobacteria decreases as we proceed from the lower to the upper intertidal zone. Accordingly, the tidal regime, salinity, elevated ambient air temperature, and desiccation periods have a great influence on the distribution of cyanobacterial community in the oil polluted intertidal zone of Abu Ali Island.

Keywords: Microbial community; Cyanobacteria mats; Oil pollution; Saudi Arabia

1. Introduction

The coastal environment of the Eastern Province of Saudi Arabia, including Abu Ali Island, is continuously subjected to oil pollution incidents as a result of damaged oil wells, oil pipeline leaks, or ballast water discharge from nearby loading terminals. This frequent oil pollution represents an additional stress factor to already harsh environmental conditions of high temperature, salinity, rapid rate of evaporation and desiccation. During the Gulf War in 1991, the Eastern Province coast of Saudi Arabia was subjected to additional contamination from oil. It was estimated that 2 million barrels of oil landed on the coastal shoreline of Saudi Arabia, severely impacting the Coast of Abu Ali Island (Barth, 2003; MEPA, 1991; Readman et al., 1992). In the last fourteen years, there have been sporadic studies focusing on coastal oil pollution (Fowler et al., 1993; Gerges, 1993; Hayes et al., 1993; Michel et al., 1993; Smith, 1996; Watt, 1996) as well as on development of cyanobacterial mats (Hoffmann, 1996; Höpner et al., 1996; Krupp et al., 1996) in various locations along the Arabian Gulf including Abu Ali Island.

Abu Ali Island is located to the northeast of the Jubail industrial city in the Arabian Gulf (Fig. 1). The northern shoreline of this island was subjected to heavy oil pollution in the 1991 oil spill, as it has played a major role in restraining the spread of spilled oil further south (MEPA, 1991; Barth, 2003). Our study site, N27.30794 E49.65179 has been subjected to moderate oil pollution due to its protected location on the southeast side of the island. It is classified as low-energy exposed beach according to the Brown and McLachlan (1990) scheme. The sediment of

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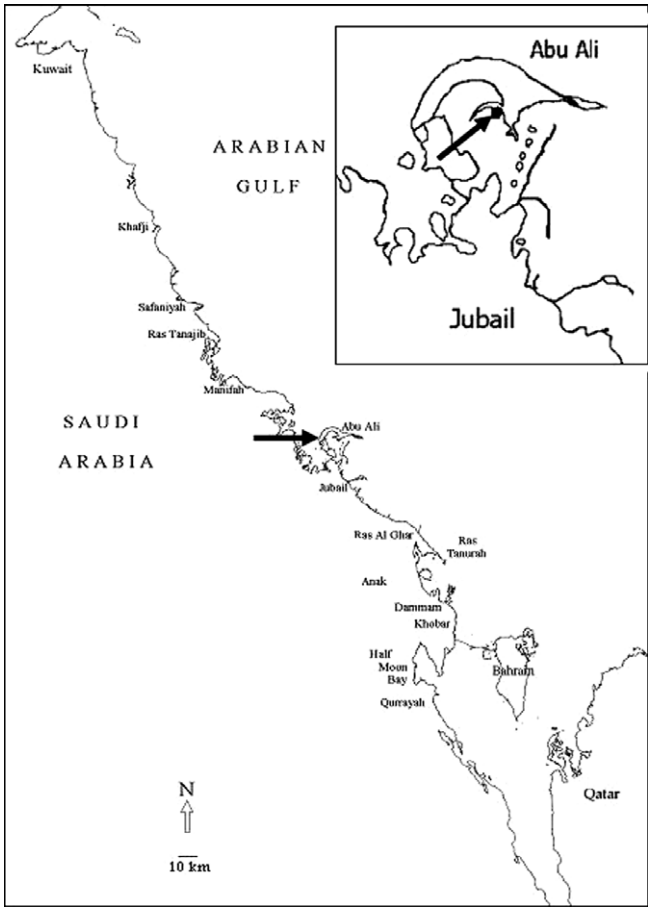

Fig. 1. Geographical location of Abu Ali Island along Saudi Arabia coast, inner map showing location of study area.

the intertidal zone consists mainly of coarse sand, oolites, and shell fragments. Cyanobacterial mats were found to cover the oil polluted sediment along the intertidal zone. Surface temperature of the exposed intertidal cyanobacterial mats during summer can reach up to 55 °C during low tide causing rapid desiccation of the cyanobacterial mat (Hoffmann, 1996). While salinity ranges from 40–45 ppt in normal subtidal condition, it can reach 60 ppt in poorly drained tide pools. Extreme environmental conditions including temperature, salinity, light intensity, and desiccation are known to be major factors in biodiversity distribution in various environments (Frontier, 1985; Atlas and Bartha, 1997). However, few studies have focused on the role of tide regime and extreme conditions on species distribution of cyanobacteria cross the intertidal zone (Rothrock and Garcia-Pichel, 2005). In this study we investigated the effect of extreme arid environmental conditions of high temperature, salinity, and periods of desiccation on cyanobacteria community distribution along various locations of oil polluted transect across the intertidal zone. We have combined traditional taxonomic and molecular techniques of Denaturing Gradient Gel Electrophoresis (DGG) to identify species composition of various genera of cyanobacteria found as part of the existing microbial consortia.

2. Methods

2.1. Samples collection and light microscopy

Mat samples were collected in April 2004 during low tide, using stainless steel core samplers. Duplicate core samples of microbial mats, 5 cm diameter and 2 cm thickness were collected along a 25 m transect across the intertidal zone (at N27.30 794, E49.65179). Samples were taken from three sites with a distance of 15 m between the upper intertidal and mid intertidal and 10 m between mid tidal and lower tidal sites (Fig. 2). Each core was divided into two equal sub-samples. One sub-sample was placed in a 200 ml sampling jar, and fixed with 3% formaldehyde in environmental sea water, to be used for light microscope analysis. The other sub-sample was placed in 100 ml sampling jar, placed in an ice box and transferred to the laboratory where it was kept at −20 °C to be used for molecular analysis. Light microscopy examination of algal mats was carried out as described by Al-Thukair and Al-Hinai (1993). Briefly, small pieces of algal mats were treated individually with 3% HCl on microscope slides, covered with cover slip and observed using Leitz, Ortholux II microscope. Thirty slides were prepared for each sampling site. Cyanobacteria and diatoms were identified to the genus level consulting Castenholz (2001), and QUIN 5 software program. Kruskal-Wallis test was used for cells measured and statistical evaluation.

2.2. Physical parameters and tidal regime

Water temperature and salinity were measured during sampling using salinity, and temperature sensors YSI Model 33, while measurements of dissolved oxygen were taken using YSI 550 A model oxygen sensor of YSI (Yellow Springs Instrument Co., Yellow Springs, Ohio 45387, USA). Briefly, calibrated probes were submerged 1 cm above the surface of the algal mats at different locations (Upper, Middle and Lower) during high tide, and readings

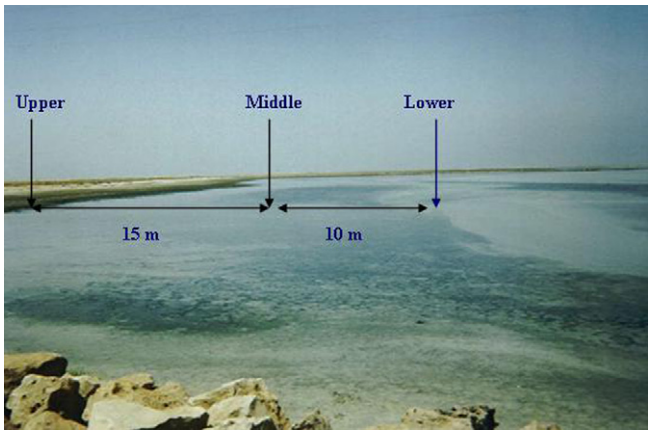

Fig. 2. Photograph of study site, and sampling location along the intertidal zone.

Table 1
Meteorological parameters, for 2005 from Jubail meteorological station

Month	Monthly mean			
	Temperature (°C)	Dew point temperature (°C)	Wind speed (mps)	Wind direction (0° N)
January	18.2	8.5	3.8	306
February	17.3	3.7	5	337
March	21.5	6.1	4.2	312
April	24.6	12.4	4.4	358
May	31.3	11.2	5	333
June	34.5	12.3	4.9	306
July	35.9	16	4.2	324
August	35.6	14.9	4.6	308
September	31.3	16	3.5	313
October	28.7	14.2	3.3	264
November	23.8	12.1	4.2	217
December	16.2	5.5	4.4	279
Annual mean	26.6	11.1	4.3	307

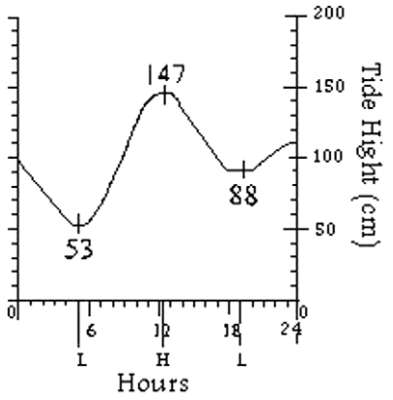

Fig. 3. Typical tide pattern for Abu Ali Island where the study site is located (L = low tide, H = high tide).

of temperature, salinity, and dissolved oxygen were recorded. Ambient temperature was recorded during sampling using a regular thermometer. Meteorological annual means for temperature, dew point, wind speed, and wind directions means were obtained for nine months from Jubail meteorological station (Table 1). Tide regime pattern of low and high tide cycles for the site was calculated from daily tide tables of Abu Ali Island. Hours of cyanobacteria mats' direct exposure to ambient air and sun light of the studied transect were calculated, and the semi-diurnal pattern was observed (Fig. 3).

2.3. Nucleic acid extraction from mats

Nucleic acids from sub-samples of mat cores were extracted as described by Abed et al. (2002). Briefly, pieces (ca. 300–500 mg each) from mats were homogenized, and subjected to freeze (in liquid N^2) and thaw (at 65 °C) cycles 3–5 times. The DNA was extracted by adding phenol–chloroform–isoamyl alcohol 25:24:1 (vol/vol/vol) and precipitated by adding 0.6 vol of isopropanol to the aqueous phase with subsequent spinning at 4500 rpm for 45 min. The pellets were suspended in 100 μl TE buffer (10 mM HCl and 1 mM EDTA at pH 8.0). Polymerase chain reaction (PCR) for the amplification of 16S rRNA was carried out using two sets of oligonucleotide primers: CYA359F (with 40 nucleotide GC clamp at the 5′ end) and CYA781R as specific primers for cyanobacteria (Nübel et al., 1997). A hot start program was performed for the cyanobacteria-specific primers as described by Nübel et al. (1997). Denaturing gradient gel electrophoresis (DGGE) was carried out as follows: Polyacrylamide gel was poured between two glass plates separated by 1 mm plastic spacers with a 20–60% urea-formamide gradient. The PCR products were applied directly to this gel and the DGGE was performed at 60 °C and a constant voltage of 200 V for 3.5 h. After electrophoresis, the gel was stained in ethidium bromide solution (0.5 μg/l) and photographed on an UV-transilluminator with a Polaroid camera. The DGGE bands were then excised, PCR reamplified, and the amplification products were sequenced. The obtained cyanobacterial 16S rRNA gene sequences were aligned and compared to available sequences in the database of the ARB software (Ludwig et al., 1998).

3. Results

At the studied site, the water level at the top of the profile during normal high tide reaches breaking waves of 10–20 cm, and 40 cm during high north winds and storms. The tide regime follows a semidiurnal pattern, with mean tide range of 0.8 m and mean spring tide of 1.35 m. Annual water temperature falls within a range of 15–37 °C, and may reach more than 40 °C in shallow isolated pools (Hoffmann, 1996). Water temperatures during our sampling were, 28.2 °C, 28.7 °C, and 29.1 °C for Lower (L), Middle (M) and Upper (U) intertidal zone respectively. Dissolved oxygen measurements were, 7.74 mg/l, 7.85 mg/l, and 8.71 mg/l, while salinity and ambient temperature for all locations was 42‰, and 32.2 °C. Meteorological data for ambient temperature, dew point, wind speed, and wind

direction for twelve months showed means of 26.6 °C, 10.6 °C, 4.8 mps and 307 0° (Table 1).

Morphologically, the mat in the Lower zone (L) is dark green, and has a smooth and thick leathery texture (5–7 mm thick). This mat is rarely exposed to air, desiccation and direct sun light. It is submerged under more than 1 m water depth during high tide. The middle zone mat (M) consists of light-brown smooth leathery layer (4–6 mm thick). It is exposed less than four hours during low tide. During high tide it is submerged under 50–60 cm of water. The mat in the upper zone (U) formed a leathery dark brown to green layer (2–4 mm thick), and in some places eroded. This mat is exposed to air and direct sun light for more than six hours during low tide. During high tide the mat is submerged under 30 cm of sea water on average. Schematic diagrams and cyanobacteria color, and texture are shown in Fig. 4.

Identification of cyanobacteria in core samples at different locations using light microscopy reveals certain differences in species compositions with different dominant forms in each zone (Fig. 5, Table 2). The morphotypes were measured for characterization, but names of morphospecies were not given. The lower zone (L) consists of *Phormidium* sp., as dominant species and other cyanobacteria species such as *Aphanothece*, *Chroococcus*, *Scytonema* and *Skeletonema* sp. as dominant diatom species. Middle zone (M) consists of *Microcoleus* sp. (probably *M.chthonoplastes*) as dominant species, accompanied by species of *Phormidium*, *Oscillatoria* and *Halothece* and diatoms (*Navacula* sp., *Amphipleura* sp. and *Mastoglia* sp.). The upper zone (U) is dominated by *Schizothrix* sp., with *Lyngbya* sp. and *Phormidium* sp., as subdominant. Diatoms include; *Navacula* sp., *Amphipleura* sp. and *Mastogloia* sp.

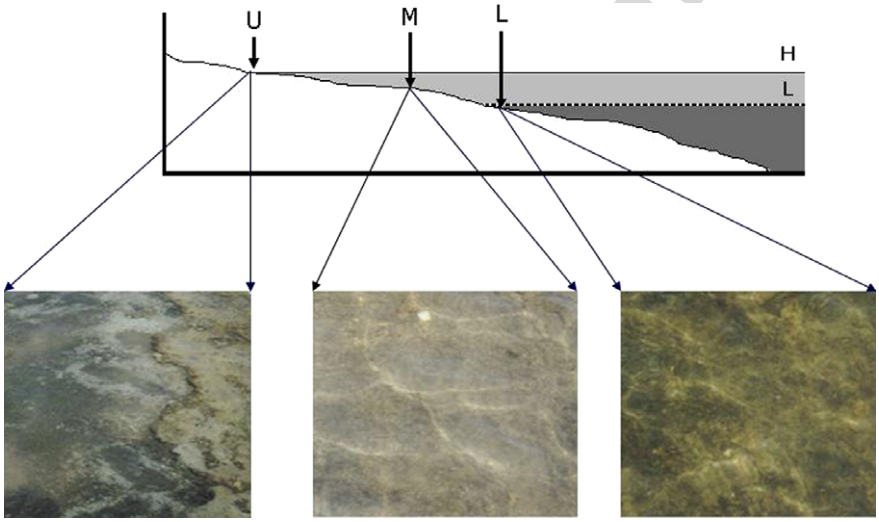

Fig. 4. Schematic diagram showing location of different types of cyanobacteria mats, and photograph of various mats along the intertidal zone (U = upper, M = middle, L = lower, H = high tide, L = low tide).

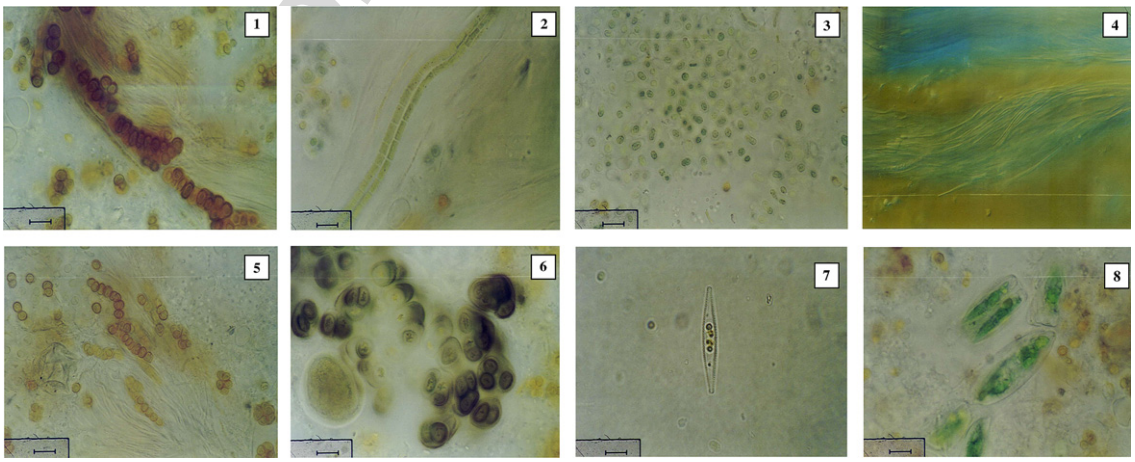

Fig. 5. Photomicrograph of various species of cyanobacteria and diatoms. (1) *Schizothrix* sp., (2) *Microcoleus* sp., (3) *Aphanothece* sp., (4) *Phormidium* sp. (Scale bar = 50 μm), (5) Thallus of *Schizothrix* sp., where filaments are arranged parallel, and surrounded with colorless sheath, (6) *Chroococcus* sp., (7) *Amphipleura* sp. and (8) *Navacula* sp. (Scale bar = 10 μm for 1, 2, 3, 5, 6, 7, 8.)

Table 2
Morphological identification of cyanobacteria and diatom species found in upper, middle and lower intertidal zone

Microorganism	Cell diameter μm length/width	Lower	Middle	Upper
Cyanobacteria				
Aphanotheca sp.	2.5–4/1.5–2	+	–	–
Chroococcus sp.	4.1–4.5/2.9–4	+	–	–
Halotheca sp.	3–6/2–5	–	+	–
Lyngbya sp.	2–3/12–16	–	–	+
Micocoleus sp.	2–4/1.5–3	–	++	–
Oscillatoria sp.	3.1–7/3–6	–	+	–
Phormidium sp.	3.2–4/4–6	++	+	+
Schizothrix sp.	1.5–3/2–3	–	–	++
Scytonema sp.	3–5/6–8	+	–	–
Diatom				
Amphipleura sp.	20–25/4–5	–	+	+
Mastogloia sp.	20–24/12–14	–	+	+
Navacula sp.	22–27/10–12	–	–	+
Skeletonema sp.	4–7/5–6	+	–	–

++: Dominant species, +: present, –: absent.

DGGE fingerprinting analysis of cyanobacterial samples taken from lower, middle and upper level of the intertidal zone using cyanobacteria-specific primers showed 8, 6 and 5 distinct bands, respectively. The number of bands (i.e. species) is highest in the sample closest to the sea and decreases across the intertidal zone upwards (Fig. 6). Analysis of 16S RNA and construction of phylogeny tree for cyanobacterial species shows that DGG bands of L7, L8, U5, M4, M5, U3, M6 fell next to assigned genera of *Oscillatoria*, *Lyngbya*, and *Microcoleus*. In addition DGG bands L4, L6, M2 and L5 fell close to the strains Cyanothece PCC7418, Euthalothece MPI 95AH13, and Dactylococcopsis PCC8305. However, the sequence of all above bands was at least 9% dissimilar when compared with those strains. DGG band L2 is close to *Phormidium minutum* D5 and DGG band U3 plotted close to *Dactylococcopsis* PC8305. DGG band L1 probably belonged to a diatom because it mapped phylogenetically close to the sequence of *Skelotonema pseudocostatum* (see Fig. 7).

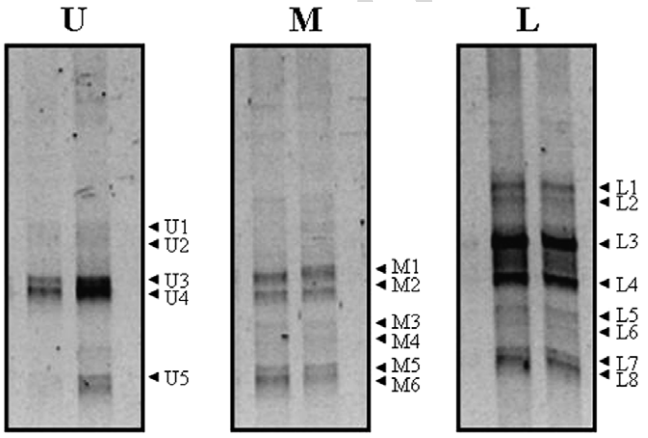

Fig. 6. DGGE profile of PCR-amplified 16S rRNA fragments obtained by using cyanobacteria primer (U = upper, M = middle, L = lower). The labeled fragments were excised, re-amplified and sequenced.

4. Discussion

Contributions of cyanobacteria in the formation of microbial mats and their role in the food chain of the intertidal zone have been investigated in various studies (Javor and Castenholz, 1984; Farmer, 1992; Whitton and Potts, 2000). Their role and contributions in the process of oil biodegradation has been of particular interest (Sorkhoh et al., 1992; Sorkhoh et al., 1995). From these studies, we could conclude that cyanobacteria are considered to be significant contributors to the food chain, and play a major role in oil biodegradation in the coastal intertidal zone.

The intertidal zone, where our study site is located, was polluted with oil during the 1991 oil spill and no remediation or clean-up procedures have taken place since that time. The entire coast within the range of more than 1 km from our site was left for self-remediation. Soon after the 1991 catastrophic oil spill, microbial mats consisting of cyanobacterial species were found to colonize oil polluted sediment of the intertidal zone (Al-Thukair and Al-Hinai, 1993; Höpner et al., 1996; Krupp et al., 1996). Field observations following the 1991 oil spill revealed an unusual "peeling off" of newly developed cyanobacterial mats in the intertidal zone, which contributed to the removal of oil through a combination of mechanical and biological degradation processes.

A six stage process was described to explain the phenomena that persists up to date (Al-Thukair, 1999). Briefly, cyanobacterial mats formed on top of oil-soaked sediments of the intertidal zone. Thereafter, cracks were formed in the mats as a result of shrinkage due to high rates of evaporation. Polygon-shaped pieces of 100 cm^2 to 1 m^2 in size were formed in the upper and middle intertidal zone, exposing the sediments beneath the mats to weathering. These mat pieces were eventually peeled off by wind and tidal currents, followed by exposure of sediments and colonization by a second generation of microbial communities that also included cyanobacteria. It has been reported that oil polluted sites covered by cyanobacterial mats showed more recovery signs than those uncovered, indicating their capacity to degrade oil (Sorkhoh et al., 1992; Sorkhoh et al., 1995; Hoffmann, 1996; Höpner et al., 1996).

Other studies have also indicated that pre-exposure to high levels of petroleum hydrocarbons resulted in a microbial community, including cyanobacteria that is adapted to hydrocarbons and contains correspondingly higher concentrations of hydrocarbon-degrading heterotrophic bacteria (Abed et al., 2002). Photosynthetic microorganisms, including cyanobacteria such as *Anabaena cylindrica*, *Phormidium foveolarum*, and *Oscillatoria* sp. play a direct or indirect role in the metabolism and degradation of different hydrocarbon compounds (Cerniglia et al., 1980; Radwan and Al-Hassan, 2000).

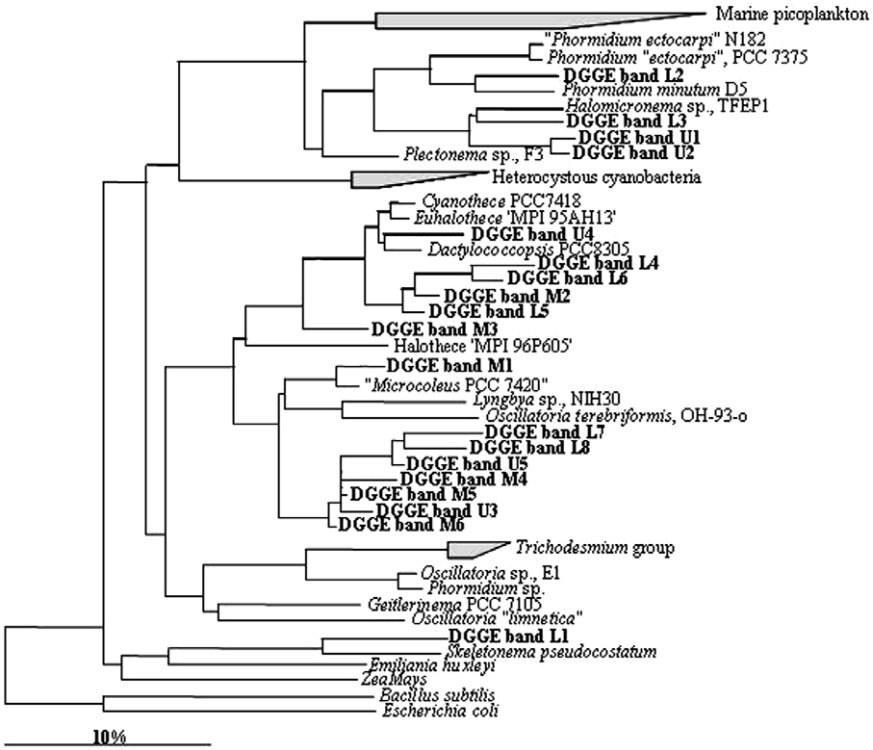

Fig. 7. Phylogenetic tree based on 16S rRNA gene sequence data showing the affiliation of predominant cyanobacteria from different location along the intertidal zone. Sequences in this study are shown in bold. The scale bar represents 10% estimated sequence divergence.

In our study site, we have observed that the cyanobacterial mats today were less developed and had thinner layers than those formed a decade ago (Al-Thukair and Al-Hinai, 1993; Hoffmann, 1996). Cyanobacterial consortia growths in our oil polluted site are subjected to harsh arid environmental conditions and dramatic daily and seasonal fluctuations of salinity, temperature, and spells of extended desiccation periods. *Lyngbya* morphotypes and those of other cyanobacterial genera, such as *Calothrix* and *Microcoleus* were reported to be dominant in the intertidal zone and have capabilities to resist desiccation (Rothrock and Garcia-Pichel, 2005). Other species of cyanobacteria such as *Anabaena* sp. were reported to have stress-responsive proteins (Apte, 2001; Rajaram and Apte, 2003).

Our study indicates that those cyanobacteria found to prevail in our site are all well adapted to oil pollution. However, their dominance shifted from the lower to the upper intertidal zone, indicating differences in tolerance to high temperature, salinity and length of desiccation periods. Since cyanobacteria in these habitats provide the basis of microbial consortia of the oil polluted intertidal zone, we expect that these different consortia may also harbor interesting and different oil degrading bacteria with different capabilities of tolerating harsh arid environmental conditions of temperature and salinity. Other studies conducted under different environmental conditions support this conclusion (Wrenn et al., 1997; Margesin and Schinner, 2001; Yakimov et al., 2004). Therefore, further studies on diversity and degradation capabilities of cyanobacteria species and associated microbial consortia within this type of mats should be considered.

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