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Spatial–temporal variations and diversity of the bacterioplankton communities in the coastal waters of Kuwait

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ABSTRACT

The dynamics and composition of the bacterial community in the coastal waters of Kuwait are poorly understood. In this study, the spatial–temporal variations in the bacterial composition in the surface water along the Kuwait coast was examined by 16S rRNA denaturing gradient gel electrophoresis (DGGE) fingerprinting and phylogeny analyses. The sampling sites were Kuwait Bay, Al-Sabbiya (north of the bay) and Al-Khairan (to the south). The bacterial composition was more variable in the summer for all sites. A cluster analysis of the DGGE fingerprint revealed two main clusters, indicating a temporal similarity between sites. Kuwait Bay and Al-Khairan were more similar to each other than to Al-Sabbiya. The bacterial community composition exhibited distinctive spatial variations, with more diversity at Al-Khairan and less diversity at Al-Sabbiya. At all sites, the dominant bacteria were Alphaproteobacteria, in particular Rhodobacteraceae, followed by Alteromonadaceae (Gammaproteobacteria) and Bacteroidetes.

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1. Introduction

The marine environment of Kuwait is situated at the western edge of the northern part of the Arabian Gulf. Kuwaiti waters are shallow, with a maximum depth of approximately 30 m (Khalaf et al., 1982). The coastal habitats of Kuwait range from exposed beaches to rocky highland, and sand silt and clay exist in both the intertidal and subtidal littoral zones (Al-Nafisi et al., 2009). Kuwaiti waters are characterized by a salinity gradient from north to south due to the diluting influence of the fresh water inflow from Shatt Al-Arab River, north of the Arabian Gulf. The water column is generally well mixed year-round, and the waters are generally oxygen-rich (Al-Yamani et al., 2004). The water temperature reaches its highest value during summer; however, the mean annual temperature of water is 23.8 °C (Dames and Moore, 1983). The mean pH is 8.2, with no significant seasonal variation. The settling of suspended matter from river discharge and dust storms dramatically affects the turbidity levels of the water column. The turbidity levels are extremely high at the north side, but decline dramatically toward the south (Al-Ghadban and El-Sammak, 2005; Al-Enezi et al., 2014). Kuwait waters are generally nutrient-rich, with high biological productivity (Al-Yamani, 2001). The inflow from Shatt Al-Arab, sewage discharge, and land runoff have a major influence on the hydrodynamics, water quality, and, most importantly, the biological productivity of the northern part of the Arabian Gulf (Al-Yamani et al., 2004).

The physicochemical characteristics, circulation, and geomorphology of Kuwaiti waters have been thoroughly examined; nevertheless, little is known about the bacterioplankton communities in these waters. Very few studies have focused on the abundance of bacterioplankton in the Kuwait marine environment. The bacterioplankton abundance was high throughout Kuwaiti waters, with an average of 3.18×10^6 cells/ml (Al-Yamani et al., 1997). This reflects the well-mixed nature of Kuwaiti waters and the northwestern Arabian Gulf, which was also found to have an average bacterioplankton abundance of 1.73×10^6 cells/ml (Al-Rifaie et al., 2008).

Marine bacterioplankton are the major drivers in the biogeochemical processes in aquatic ecosystems (Kent et al., 2007; Pomeroy et al., 2007). Bacterioplankton facilitate the cycling of organic matter and nutrients by maintaining the marine ecosystem's health, balance, and ability to recover from damage. Marine bacterial populations are usually complex and often contain unidentified or uncultivated members (Pace, 1997). In addition, marine bacteria exhibit a seasonal diversity pattern in pelagic ecosystems, showing a higher diversity during winter than summer (Fuhrman et al., 2006). The bacterioplankton abundance and diversity in the marine environment is regulated by numerous environmental factors, such as temperature, salinity, dissolved oxygen levels and nutrient availability (Fuhrman et al., 2006; Gilbert et al., 2009).

The coastal waters of Kuwait are severely stressed by marine pollution, the main sources of which are petroleum-related industries, urbanization, industrial expansion, municipal wastewater, effluent from desalination and power plants, urban runoff, and the increase in marine-based recreation (Al-Muzaini, 2002; Al-Yamani et al., 2006).

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The major pumping failure at the Mishref sewage station in 2009 released approximately 180,000–200,000 m³/day of untreated sewage into the coastal environment for several months, and the sewage discharged into the Kuwaiti coastal waters was usually high in organic content (Ghannoum et al., 1991; Al-Ghadban et al., 2002). Sewage discharge may cause coastal eutrophication, the accumulation of toxic substances in coastal sediments and support the growth of hazardous algal blooms.

This study aims to examine the seasonal spatiotemporal patterns of bacterioplankton dynamics in the surface water at three areas along the coast of Kuwait to gain a deeper insight into the microbial ecology of this more impacted marine environment. The diversity of the bacterial community will also be explored to gain an overall understanding of the structure of the bacterioplankton in these waters and the effect of various environmental factors on modulating the bacterial community structure in Kuwaiti waters.

2. Materials and methods

2.1. Study site and sampling

The water samples were collected from three sites (Fig. 1). The northernmost site was Khor Al-Sabbiya (N29.37.133, E48.09.099), a long submerged estuarine channel located on the northern coast of Kuwait. Al-Sabbiya is a muddy intertidal flat that is characterized by a plume of suspended sediments. The second site was Kuwait Bay, an elliptical embayment at the northwest corner of the Arabian Gulf and north of Kuwait city (N29.19.429, E47.52.774). Kuwait Bay is a semi-enclosed, nonestuarine environment with an area of approximately 750 km² (Al-Ghadban and El-Sammak, 2005). The water is shallow throughout, with an average depth of approximately 8 m and a maximum depth of less than 15 m (Al-Yamani et al., 2004). Kuwait Bay has soft sediments, slow tidal currents, high turbidity, little sediment transport, and the bay has undergone considerable development and is almost completely urbanized. The third site is Al-Khairan, on the southern coast of Kuwait (N28.32.820, E48.28.144). South of Kuwait Bay, the coast is dominated by sand beaches that are relatively unprotected from the open Gulf waves, and almost the entire south coast has been anthropogenically modified.

Sampling was conducted every 2 months from April 2010 until February 2011. A 1-L sample of surface seawater was collected in a clean container at each site. The samples were placed in a cooler to maintain their low temperature and were immediately transported to the laboratory. At the same time that the samples were collected, the ambient water temperature, salinity, pH, turbidity, and dissolved oxygen content of the surface water were measured directly at the site using a Water Quality Checker U-10 (HORIBA). In the lab, the samples were pre-filtered through a 47-mm-diameter polycarbonate filter (nominal pore size 3 µm) to remove the large particles and then filtered through a 0.22-µm PES filter (Nalgene). The filters were then immediately placed in sterile containers and stored at –20 °C before being processed. The chlorophyll *a*, total organic carbon, petroleum hydrocarbons and nutrient measurements were obtained from the annual statistical bulletin of the environment published by the Kuwait Environmental Public Authority (KEPA).

2.2. DNA extraction

Under sterile conditions, the total genomic DNA was extracted from the filters. Each sample manipulation was performed separately to avoid cross-contamination. The DNA was extracted from frozen filters using a FastDNA spin kit for soil (MP Biomedical) according to the manufacturer's instructions. The integrity of the extracted DNA was verified by electrophoresis on a 0.8% agarose gel in 1 × TAE buffer.

2.3. Polymerase chain reaction (PCR) amplification

The 16S rRNA genes were amplified from the DNA templates by the Ready-to-Go PCR beads (GE Healthcare, UK) using the universal bacterial primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTACCTGTACGACTT-3') (Lane, 1991). PCR was performed in 25-µL reaction mixtures (0.5 µM of each primer and 1 µL of the DNA template). The cycling conditions were 94 °C for 5 min, followed by 30 cycles of 94 °C for 1 min and 55 °C for 1 min, with a final extension at 72 °C for 10 min. The amplified DNA was diluted by a factor of 100 and used as a template for a second PCR amplification to amplify the 590-bp DNA fragments of the V3 region of the 16S rRNA using the primers GM5F (5'-CCTACGGGAGGCAGCAG-3') and 907R (5'-CCGTC



Fig. 1. Map of Kuwait showing the three sampling sites.

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