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Bacterial diversity of polluted surface sediments in the northern Adriatic Sea

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ABSTRACT

Samples were collected from sea sediments at seven sites in the northern Adriatic Sea that included six sites next to industrial complexes and one from a tourist site (recreational beach). The samples were assayed for alkanes and polycyclic aromatic hydrocarbons. The composition of the hydrocarbon samples suggested that industrial pollution was present in most cases. A sample from one site was also grown aerobically under crude oil enrichment in order to evaluate the response of indigenous bacterial populations to crude oil exposure. Analysis of 16S rRNA gene sequences showed varying microbial biodiversity depending on the level of pollution – ranging from low (200 detected genera) to high (1000+ genera) biodiversity, with lowest biodiversity observed in polluted samples. This indicated that there was considerable biodiversity in all sediment samples but it was severely restricted after exposure to crude oil selection pressure. Phylogenetic analysis of putative *alkB* genes showed high evolutionary diversity of the enzymes in the samples and suggested great potential for bioremediation and bioprospecting. The first systematic analysis of bacterial communities from sediments of the northern Adriatic Sea is presented, and it will provide a baseline assessment that may serve as a reference point for ecosystem changes and hydrocarbon degrading potential – a potential that could soon gain importance due to plans for oil exploitation in the area.

Introduction

More than one third of the human population lives in coastal areas and on small islands. Anthropogenic contamination and accidental oil spills are a major source of marine pollution that cause huge damage to the environment, resulting in extensive and long-term deterioration of coastal ecosystems. Natural oil seepage and marine oil transport accidents provide a constant input of pollutants to marine environments. Research on microbial alkane degradation started a century ago, with a publication by Söhngen [42] on microorganisms responsible for the disappearance of oil spills on surface waters. Many different enzymes involved in hydrocarbon degradation have been characterized to date and almost 200 bacterial, cyanobacterial, algal and fungal genera, representing more than 500 species, are known to thrive on hydrocarbons. Crude oil is perhaps one of the most complex mixtures of organic compounds on Earth that is typically high in saturated and aromatic hydrocarbons [40]. Alkanes are rather inert chemical substances

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and the first stage in their biological degradation is an activation step. In aerobic degradation of linear alkanes, the activating enzyme is often a monooxygenase, which produces a terminal alcohol group. A variety of monooxygenases are known that have different substrate specificities for short (C_2–C_4), medium (C_5–C_{17}) and long chains (>C_{18}). One of the most intensively investigated groups of enzymes is the family of AlkB integral membrane non-heme iron monooxygenases, which are common in strains degrading medium chain length alkanes [40].

Alkane degrading microorganisms are usually highly enriched in samples from polluted sites, and some recently characterized bacterial species have been shown to be highly specialized for hydrocarbon degradation. These species are called obligate hydrocarbonoclastic bacteria, and they play a key role in the removal of hydrocarbons from polluted and non-polluted environments [40,48,50].

Degradation of pollutants in contaminated environments is, in many cases, carried out by microbial food webs rather than by a single species. As a large proportion of environmental microorganisms cannot be grown in the laboratory, a proper understanding of the microbial communities present can only be gained by analysing the metagenome (i.e. characterising the DNA sequences of all the organisms present in the sample). In the last decade, functional genomics technologies have been introduced into research on microbial alkane degradation [45].

The coastal region of the Kvarner Gulf and the Istrian Peninsula are the best-developed tourist regions in the northern Adriatic Sea. However, the oldest shipyard and oil refinery on the Croatian coast are located in the two most important cities (Pula and Rijeka). The INA oil refinery is a medium-sized refinery located in Kvarner Bay near the city of Rijeka. The refinery processes approximately 3.5 million tons of crude oil annually and manufactures a large number of petroleum products. The Uljanik shipyard in Pula Bay (est. 1856) is the most significant shipyard in the Croatian part of the Adriatic Sea.

Little is known about the distribution of polluting hydrocarbons in the region and its effect on microbial populations. The first analyses of PAHs in marine sediments within Rijeka Bay started in 1998 at three sampling sites offshore from the petroleum refinery facilities, and they were extended in 1999 to three more sampling points in front of the repair shipyard within the same eastern industrial zone [2]. The concentrations of PAHs were considerably higher in the shipyard environment in comparison with the petroleum refinery area. A declining trend of total PAHs and consequent toxicity indices was observed at all sites [2]. In a recent study by Treven [44], historical trends (2007–2012) of polycyclic aromatic hydrocarbon (PAH) pollution in surface sediments in the northern Adriatic Sea (Croatia) were assessed, and the results indicated a relatively low concentration of PAHs in marine sediments in the proximity of the oil refinery located in Kvarner Bay. Proposals to exploit oil and gas reserves in the Adriatic make it even more important to establish the present distribution of pollution and to prepare for likely further pollution associated with new industries.

For this study, seven sampling points of coastal surface sea sediments were investigated. Six of the sites were subject to long-term industrial contamination due to the proximity of major industrial complexes and the other site in Svezanj Cove was chosen as a "clean" point. In addition, one of the samples was subjected to enrichment with crude oil (SF1). The hydrocarbon compositions of the samples were analysed by gas chromatography/mass spectrometry. The composition of the bacterial populations was determined by analysis of 16S rRNA sequences, and the occurrence of the *alkB* gene was used as a marker for the presence of alkane-degrading bacteria. These studies formed the basis for further metagenomic studies.

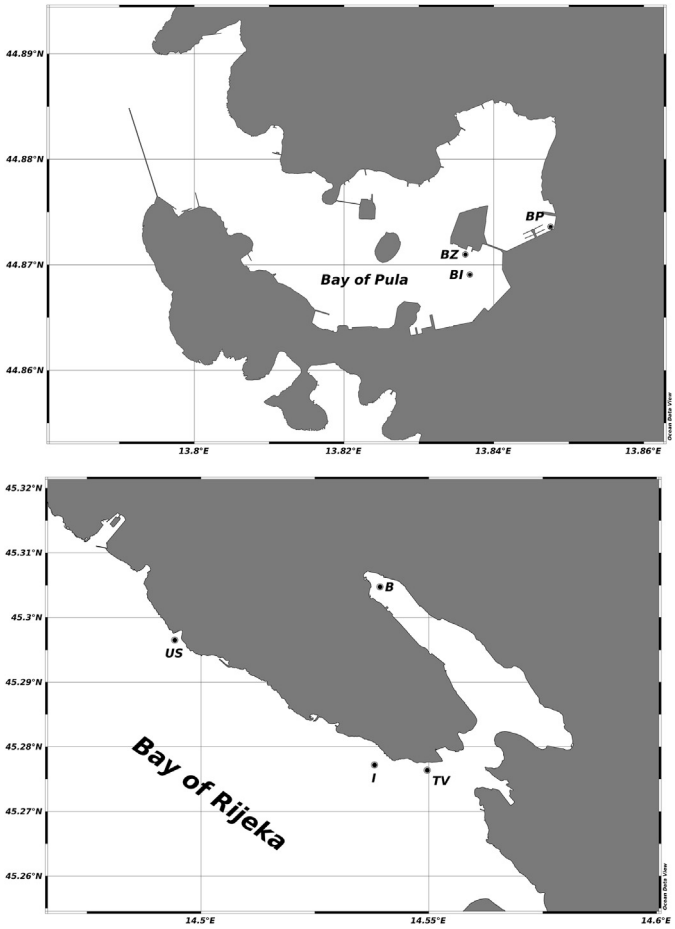

Fig. 1. Research area and sampling stations in the northern Adriatic Sea.

Materials and methods

Area description, sampling locations and processing

The coastal surface sediment samples (0–3 cm depth) were collected from seven locations within Rijeka and Pula Bays in May 2012. The exact locations are shown in Fig. 1. The stations in Rijeka Bay (sandy sediments, average 60 m depth) were facing a former coke plant area (B; latitude: 45.304745, longitude: 14.539268), petroleum refinery facilities (I; latitude: 45.277181, longitude: 14.538066) and a tanker berth (TV; latitude: 45.276365, longitude: 14.549654). The stations in Pula Bay (clay sediments, average 15 m depth) were facing a marine gas station (BP; latitude: 44.873499, longitude: 13.847572), and Uljanik repair shipyard's vent (BI; latitude: 44.869053, longitude: 13.836787) and cove (BZ; latitude: 44.870969, longitude: 13.836186). As a reference point, a sample was also taken from Svežanj Cove (US; latitude: 45.296504, longitude: 14.494250) and represented a touristic recreational zone that should not be polluted by oil spills. A SCUBA diver collected the sediment samples manually with gravity core samplers. The sediment samples were freeze-dried for 72 h and fully homogenized. The coastal sediment from the tanker berth station (TV) was subsampled into a sterile Erlenmeyer flask and grown under crude oil pressure in aerobic (SF1) conditions using the method described by Wang et al. [47] for four months before the nucleic acids were extracted and sequenced (Table 1) (Fig. 2).