Marine Pollution Bulletin xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Hydrocarbon pollutants shape bacterial community assembly of harbor sediments

Marta Barbato [a,1], Francesca Mapelli [a,1], Mirko Magagnini [b], Bessem Chouaia [a,2], Monica Armeni [b], Ramona Marasco [c], Elena Crotti [a], Daniele Daffonchio [a,c], Sara Borin [a,*]

[a] Department of Food, Environmental and Nutritional Sciences (DeFENS), University of Milan, Milan, Italy
[b] EcoTechSystems Ltd., Ancona, Italy
[c] Biological and Environmental Sciences and Engineering Division (BESE), King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900, Saudi Arabia

ARTICLE INFO

Article history:
Received 6 August 2015
Received in revised form 19 January 2016
Accepted 20 January 2016
Available online xxxx

Keywords:
Oil contamination
Autochthonous bioaugmentation
Pyrosequencing
Bacterial community
Alcanivorax

ABSTRACT

Petroleum pollution results in co-contamination by different classes of molecules, entailing the occurrence of marine sediments difficult to remediate, as in the case of the Ancona harbor (Mediterranean Sea, Italy). Autochthonous bioaugmentation (ABA), by exploiting the indigenous microbes of the environment to be treated, could represent a successful bioremediation strategy. In this perspective we aimed to i) identify the main drivers of the bacterial communities' richness in the sediments, ii) establish enrichment cultures with different hydrocarbon pollutants evaluating their effects on the bacterial communities' composition, and iii) obtain a collection of hydrocarbon degrading bacteria potentially exploitable in ABA. The correlation between the selection of different specialized bacterial populations and the type of pollutants was demonstrated by culture-independent analyses, and by establishing a collection of bacteria with different hydrocarbon degradation traits. Our observations indicate that pollution dictates the diversity of sediment bacterial communities and shapes the ABA potential in harbor sediments.

1. Introduction

The Mediterranean Sea is one of the most congested basins in terms of oil tanker traffic, hosting about 20% of the global traffic. Furthermore the coastlines are densely inhabited and, especially in the northern side, highly industrialized. As a consequence, Mediterranean coasts and harbors, especially the industrial ones, are constantly exposed to crude oil hydrocarbons (HC) deriving mainly from oil tankers and wastes of refineries, chemical industries and oil pipelines (Daffonchio et al., 2012, 2013). The thousands of different oil molecules have a wide range of physico-chemical properties and toxicity. The individual molecules within the four main groups of oil HC, saturated HC, aromatic HC, resins and asphaltenes, are also classified according to their molecular weight into light fractions (low molecular weight) and heavy fractions (high molecular weight). The latter includes the most recalcitrant compounds to degradation that tend to accumulate in the sediments of harbors and coastal areas nearby oil-related facilities.

The Ancona harbor (Italy) is located in the Adriatic Sea and is one of the largest Mediterranean commercial harbors, with intense ferry and merchant fluxes, and it is surrounded by shipyards and important industrial plants. High concentration of pollutant chemicals, including aliphatic HC, polycyclic aromatic HC and naphthalene have been measured in its sediments, along with high concentration of different heavy metals like Cu, Mn, Ni and Zn (Dell'Anno et al., 2009; Mei and Danovaro, 2004; Mirto and Danovaro, 2004).

Bioaugmentation is the addition of degrading bacteria to a polluted environment and it is recognized as a potential strategy for supporting the clean-up of polluted sediments (Head et al., 2006). We have recently shown that the origin of the added microorganisms is essential to predict the success of a sediment treatment through bioaugmentation and that allochthonous microorganisms present several limitations due to the adaptation to the local environmental conditions (Fodelianakis et al., 2015). In this perspective, a new approach for bioaugmentation, called autochthonous bioaugmentation (ABA), has been proposed (Hosokawa et al., 2009) and recognized as the best strategy for remediate marine oil polluted samples (Nikolopoulou et al., 2013). ABA is a site-tailored strategy based on the use of autochthonous microbial populations, previously enriched under laboratory conditions and re-inoculated in the polluted site to be treated, taking advantage of their natural ability to cope with the unique environmental setting where they come from. To set up successful ABA strategy, it is pivotal to gain knowledge of the indigenous microbial populations, of the occurring environmental parameters and their influence on survival and degradation rate of the selected microorganisms.

The present study aimed to assess i) the driving factors shaping the bacterial communities inhabiting the Ancona harbor's sediments through culture-dependent and independent approaches and ii) the

* Corresponding author.
E-mail address: sara.borin@unimi.it (S. Borin).
[1] These authors equally contributed to this work.
[2] Actual address: Department of Entomology, 5142 Comstock Hall, Cornell University, Ithaca, NY 14853, United States.

http://dx.doi.org/10.1016/j.marpolbul.2016.01.029
0025-326X/

effect of different HC pollutants on the bacterial community richness and diversity.

2. Materials and methods

2.1. Site description and sampling and sediment physico-chemical characterization

Sediment samples were collected from ten polluted stations in the harbor (codes: U1–U10) and five control pristine stations (codes: C1–C5) 3.5 km far away from the harbor, along the dominant NW/SE current (Table 1, Fig. 1a). Sampling depths ranged from 2.5 m in the harbor area to 13 m in the control stations.

For each sample, pH and redox potential were measured. Samples for granulometric analyses (percentages of gravel, sand and clay in the sediment), humidity and specific weight were transferred into plastic containers and stored at +4 °C. For the analyses of trace metals, HC and total polycyclic aromatic hydrocarbons (PAHs), samples were transferred into inert HDPE containers and stored at −18 °C until laboratory analyses. Samples for the determination of total organic carbon (TOC) and molecular ecology analyses were transferred, respectively, into Petri dishes and sterile plastic containers, and stored at −20 °C. For the determination of total bacteria, sediment slurries were prepared using sterile buffered formaldehyde (4% final concentration), while for bacteria isolation sediment sub-samples were collected using a sterile spoon and stored at +4 °C.

TOC was determined by weight loss on combustion technique after removal of carbonate with dilute (IN) HCl. A portion of sediments was weighed into a porcelain crucible and ignited in a muffle furnace at 550 °C for 2 h. The crucible was cooled in a desiccator, re-weighed and the TOC was calculated as the weight loss in percentage (Gaudette et al., 1974). The presence and abundance of different HCs – C>12 and PAHs – was estimated by chromatographic techniques. The HC concentration was analyzed by Agilent 7890 -USA. A HP-5 capillary chromatographic column (30 m × 0.32 mm I.D.) and a capillary column (30 m × 0.25 mm I.D.) were used for GC–FID and GC–MS analyses, respectively. Nitrogen was the carrier gas with 3 mL/min. Injector and detector temperatures were maintained at 300 °C and 320 °C, respectively. The identification of n-paraffin peaks was established using a reference mixture of n-paraffin of known composition.

To determine the total content of heavy metals (Cu, Zn, Cr, Ni, Cd, Pb, Hg, V and As) in sediments, samples (0.1 g) were digested with HNO_3/$HClO_4$ (4:1, v/v) in a microwave oven (CEM, MARS5). After digestion, the volume of each sample was adjusted to 20 mL using deionized water. Heavy metals and As content were determined by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent Technologies, Santa Clara, CA, USA). Standards of heavy metals and of As for concentrations ranging from 0 to 1 mg/L were prepared from multi-element calibration standard-2A solution (Agilent Technologies) and from sodium arsenite solution ($NaAsO_2$) (Sigma-Aldrich, St Louis, MO, USA) respectively. For all the measures by ICP-MS an aliquot of a 2 mg/L of an internal standard solution (45Sc, 89Y, 159Tb, Agilent Technologies) was added both to samples and a calibration curve to give a final concentration of 20 μg/L. The instrument was tuned daily with a multi-element tuning solution for optimized signal-to-noise ratio.

2.2. Total prokaryote and hydrocarbonoclastic bacteria abundance

Total prokaryotic abundance was determined on sediment collected in the surface layer (0–1 cm). The samples were suspended in pyrophosphate solution (final concentration 5 mM) and sonicated (60 W, 1 min for three consecutive times, with 30-second intervals). The supernatant was then sub-sampled and filtered into sterile 0.2 μm pore size filters. Filters were then stained with SYBR Green I and cells were counted by means of an epifluorescence microscope (EFM) at 1000× magnification.

The number of HC-degrading bacteria in the U5 and U7 sites was evaluated through the most probable number (MPN) method, modifying the previously described method (Cappello et al., 2006). Serial dilutions of the sediments were performed and inoculated 1:10 in triplicates in ONR7a mineral medium (Dyksterhouse et al., 1995) added with crude oil (1% v/v) as unique carbon source and cycloheximide (0.01% w/v) to inhibit eukaryotic growth. Bacterial growth was evaluated after 28 days static incubation at 25 °C.

2.3. Bacterial ARISA fingerprinting and community 16S rRNA gene profiling and sequencing

Total DNA was extracted from 0.5 g of sediment using the "Power Soil" kit (MoBio Laboratories Inc., Carlsbad, CA, USA) following the manufacturer's instructions. DNA was quantified using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA).

ARISA-PCR was conducted on 20 ng of DNA template on each sample by using the primer set ITSF, 5′-GTC GTA ACA AGG TAG GCC GTA-3′ and ITSReub, 5′-GCC AAG GCA TCC ACC 3′, as previously described (Cardinale et al., 2004). ARISA fragments were separated by using the ABI3730XL genetic analyzer applying the internal standard 1200-LIZ. The output peak matrix was transferred to Microsoft Excel for the following analysis. Peaks showing a height value of <50 were removed from the output peak matrix before statistical analyses. Each polymorphic ARISA peak is defined as a different OTU. Richness was defined as the number of OTUs present in each sample.

Barcoded pyrosequencing assays on the 16S rRNA gene were carried out using bacterial universal primers (27 F mod 5′-

Table 1
Physical characterization and value of total prokaryotic abundance of the surface sediments collected at the Ancona harbor.

Station name	Sampling coordinates		Physical features						Prokaryotic cell number (±SDT)	
	Latitude	Longitude	Gravel (%)	Sand (%)	Silt (%)	Clay (%)	pH	Eh (mV)		
U-1	43°37′28.98″ N	13°29′59.85″E	0	17.67	23.23	59.1	7.56	179.1	7.48E+08	(±2.21E+07)
U-2	43°37′25.91″ N	13°30′12.21″E	3.42	15.68	18.26	62.7	7.7	197	7.16E+08	(±3.78E+07)
U-3	43°37′27.55″ N	13°30′13.07″E	2.92	15.62	19.96	61.5	7.75	137.4	6.14E+08	(±8.90E+07)
U-4	43°36′47.25″ N	13°30′10.17″E	0	18.51	33.1	48.4	7.76	247.3	1.10E+09	(±2.01E+08)
U-5	43°36′55.16″ N	13°30′9.98″E	0	24.07	27.98	47.9	7.77	43.2	1.13E+09	(±1.70E+08)
U-6	43°37′1.63″ N	13°30′10.37″E	0	25.59	25.14	49.3	7.8	161.4	8.25E+08	(±2.72E+08)
U-7	43°37′22.63″ N	13°30′13.64″E	2.53	8.3	23.98	65.2	7.72	128.6	4.04E+08	(±3.68E+07)
U-8	43°36′53.25″ N	13°29′36.60″E	0	12.75	24.15	63.1	7.64	121.4	7.04E+08	(±2.85E+08)
U-9	43°37′36.05″ N	13°29′37.41″E	0	21.42	32.33	46.3	7.85	28	7.20E+08	(±3.81E+08)
U-10	43°37′52.16″ N	13°29′51.71″E	0.16	22.24	27.2	50.4	7.5	142.2	7.00E+08	(±2.34E+08)
C-1	43°39′14.84″ N	13°29′9.79″E	0	41.49	21.38	37.1	7.68	92.6	7.46E+08	(±2.11E+08)
C-2	43°39′9.52″ N	13°28′5.26″E	0	16.11	31.34	52.6	7.47	28.4	1.03E+08	(±1.99E+07)
C-3	43°38′49.39″ N	13°30′16.13″E	0	40.91	21.84	37.2	7.78	160.4	1.89E+08	(±3.02E+07)
C-4	43°38′3.74″ N	13°32′37.44″E	0	18.9	33.22	47.9	7.72	101.3	4.65E+08	(±3.14E+07)
C-5	43°38′5.46″ N	13°34′30.15″E	0	16.19	30.85	53	7.65	83.2	9.76E+07	(±2.70E+07)

Please cite this article as: Barbato, M., et al., Hydrocarbon pollutants shape bacterial community assembly of harbor sediments, Marine Pollution Bulletin (2016), http://dx.doi.org/10.1016/j.marpolbul.2016.01.029