Intrinsic bioremediation potential of a chronically polluted marine coastal area

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ABSTRACT

A microbiological survey of the Priolo Bay (eastern coast of Sicily, Ionian Sea), a chronically polluted marine coastal area, was carried out in order to discern its intrinsic bioremediation potential. Microbiological analysis, 16S rDNA-based DGGE fingerprinting and PLFAs analysis were performed on seawater and sediment samples from six stations on two transects. Higher diversity and variability among stations was detected by DGGE in sediment than in water samples although seawater revealed higher diversity of culturable hydrocarbon-degrading bacteria. The most polluted sediment hosted higher total bacterial diversity and higher abundance and diversity of culturable HC degraders. Alkane- and PAH-degrading bacteria were isolated from all stations and assigned to *Alcanivorax*, *Marinobacter*, *Thalassospira*, *Alteromonas* and *Oleibacter* (first isolation from the Mediterranean area). High total microbial diversity associated to a large selection of HC degraders is believed to contribute to natural attenuation of the area, provided that new contaminant contributions are avoided.

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1. Introduction

Petroleum hydrocarbons are among the most widespread environmental pollutants (Hassanshahian et al., 2012). Oil pollution may arise either accidentally or operationally; indeed, pollutants may enter into marine ecosystems following catastrophic accidents (shipping disasters or pipeline failures), chronic pollution (ships, ports, oil terminals, freshwater runoff, rivers and sewage systems), natural oil seepages and natural sources (biota) (Floodgate, 1972).

In many aquatic systems, sediment generally act as a sink of pollutants, which may afterwards move back up through the water column causing serious consequences. Indeed, compared to upper layers of the water column, the bottom ones are characterized by a higher concentration of pollutants because of the re-suspension of sediment and of associated pollutants (McGenity et al., 2012).

The presence of pollutants in marine sediment may produce adverse effects on marine ecosystems and human health. Benthic organisms can gather toxic substances in their body, affecting and poisoning predators at the highest level of the food chain (Moreno et al., 2013). As hydrocarbon components belong to the family of carcinogens and neurotoxic organic pollutants, harmful effects are expected on humans exposed to such contaminants (Jarvis et al., 2014).

Increased awareness of the importance of microbial communities in marine environmental processes, together with their ability to respond to environmental contamination, has intensified the attention on the relation between microbial community structure and contaminant concentration. Among the contaminants most commonly introduced into the marine environment, hydrocarbons can exert a selective pressure on microbial communities (e.g. Langworthy et al., 2002; Wang and Tam, 2012). Bacterial communities have therefore great potential to be used as sensitive indicators of contamination in marine sediment (Sun et al., 2012). On the other hand, microorganisms (bacteria and fungi) are the main degraders of organic pollutants in marine environments. Many marine bacteria endowed with biodegradative abilities have been described (Yakimov et al., 2007) and their role in the bioremediation of contaminated sites has been addressed (Ron and

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Rosenberg, 2014). Techniques of bioremediation and utilization of oil degrading bacteria and hydrocarbon-degrading microbial consortia have a key role in these processes (Beolchini et al., 2010; Rocchetti et al., 2011). In general, bioremediation is based on in situ stimulation of the microbial community (biostimulation) or amending the microbial community with an inoculum of hydrocarbon-degrading bacteria (bioaugmentation).

Biological treatments using marine bacteria are becoming more important, mainly because of the low environmental impact, cheaper costs than other clean-up technologies, the capability to degrade organic contaminants and the possibility of beneficial use of treated sediment (Rulkens and Bruning, 2005).

Polluted sediment can be sanitized via in situ actions, which always require an accurate knowledge of the local biocenosis (Doni et al., 2013). In particular, marine obligate hydrocarbonoclastic bacteria exhibit peculiar properties on hydrocarbon degradation (Yakimov et al., 2007) and, when nutrients (as nitrogen and phosphorous) are available at not limiting concentrations, an increase of their growth rate is registered with the decrease of pollutants (Röling et al., 2004; Cappello et al., 2007a, 2007b; Yakimov et al., 2006, 2007). The complexity of contaminant transformation processes in the natural environment is due to the influence of the nature and amount of hydrocarbons, the structure and dynamics of the indigenous microbial community and the interplay of geochemical and biological factors at contaminated sites (Atlas, 1981; Leahy and Colwell, 1990; Gibson and Sayler, 1992).

A better understanding of the processes inherent to natural bioremediation requires enhancement of microbial ecology awareness. The first challenge is to overcome the complicated attribution of in situ microbe-mediated processes to the responsible organism(s), improving the knowledge on dynamics and functional activity together with identification of ecologically significant strains and genes in response to environmental stressors.

Characterization of microbial communities can provide important information to understand the extent of contamination in the sedimentary compartment and in the water column, and also to assess and predict the catabolic potential of environmental sites. DNA-based molecular analysis, such as 16S rDNA-based DGGE fingerprinting (Nocker et al., 2007), and biochemical analysis of phospholipid fatty acids (PLFA), that are essential component of viable bacterial cell membranes (Findlay et al., 1990; Kaur et al., 2005), are considered as robust tools to depict the bacterial communities in relation to environmental stressors.

The Augusta Bay, on the eastern coast of Sicily (Ionian Sea) is identified as an area with heavy industrialization (petrochemical and wastewater treatment plants) and high contamination due also to the presence of the Augusta Harbour in the northern part of the bay. Navigation and dredging of polluted sediment, in addition to direct discharge of contaminants from the industries occurred during the last century, have been identified as the main cause of the environmental contamination in the harbour and nearby (Bellucci et al., 2012). Indeed, severe inorganic and organic pollution has been detected both in and outside the harbour, as effect of dumping of dredged sediment in the surrounding area (ISPRA-APAT, 2007; ISPRA, 2010; Sprovieri et al., 2012; Di Leonardo et al., 2014) with important risks on ecosystem and human health (Ausili et al., 2008; Bonsignore et al., 2013; Genovese et al., 2014).

The goals of the present study are: (i) to characterize the organic contamination of surface sediment of the southern part of Augusta Bay, Priolo Bay (Sicily, Italy); (ii) to perform a molecular and biochemical analysis of the indigenous microbial communities associated with this area and (iii) to isolate oil degrading bacteria and hydrocarbonoclastic bacteria to discern the intrinsic bioremediation potential of the area.

2. Materials and methods

2.1. Sample collection

Seawater and sediment samples were collected in Priolo Bay (South-East Sicily, Italy, Mediterranean Sea) in July 2012 (Fig. 1). Surface sediment (0–5 cm, Fig. S1) samples with the upper seawater layer were collected in triplicate using sterile Plexiglas cores (20 cm long, 5 cm diameter), from three stations located along a northern transect (N1, N2 and N3) and three stations located along a southern transect (S4, S5 and S6) (Fig. 1).

After collection, samples were immediately transported to the laboratory in a cool box (4 ± 1 °C), where samples were used for immediate analysis or aliquots were stored at −80 ± 5 °C and at −20 ± 1 °C with glycerol (20% final concentration). Unfortunately, part of the water component of core samples of station S4 was lost during sampling and could only be analyzed for PLFAs, and isolation of HC degraders. The physico-chemical characteristics of the sediment are described elsewhere (Di Leonardo et al., 2014).

2.2. Analysis of hydrocarbons

Total hydrocarbons were extracted from sediment following the 3550C EPA (Environmental Protection Agency) procedure (Rocchetti et al., 2012). Briefly, a mixture of $CH_2Cl_2:CH_3COCH_3$ (1:1, v/v) was added to sediment samples. The mixture was sonicated for 2 min in ultrasound bath (Branson 1200 Ultrasonic Cleaner, Branson, USA). Samples were further shaken at 150g for 30 min, centrifuged for 10 min at 5000g and supernatant was passed through a ceramic column filled with anhydrous Na_2SO_4 (Sigma–Aldrich, Milan). Same treatment of pooled and dried sediment was repeated with CH_2Cl_2 and the obtained extracts were combined and volatilized to dryness. Residues were re-suspended in CH_2Cl_2 prior the gas chromatography (GC) analysis. All measures were performed using a Master GC DANI Instruments (Development Analytical Instruments), equipped with SSL injector and FID detector. Samples (1 μl) were injected in splitless mode at 330 °C. The analytical column was a Restek Rxi-5 Sil MS with Integra-Guard, 30 m × 0.25 mm (ID × 0.25 μm film thickness). Helium carrier gas was maintained at a constant flow of 1.5 ml min^{-1}. Total hydrocarbons were also calculated for each sample (Genovese et al., 2014). Three samples for any stations in study were analyzed.

2.3. Phospholipids fatty acids (PLFA) extraction and analysis

Approximately 4 g of freeze-dried sediment was weighted in screw-cap Pyrex test tubes. Lipids were extracted in triplicate and separated into fractions following the method proposed by Bligh and Dyer (1959) and modified by White et al. (1979), that is with a mixture of chloroform:methanol:P-buffer solution (1:2:0.8 v/v/v). Phospholipids were separated from the other lipid fractions using silicic solid phase extraction (SPE) cartridges under low vacuum and derivatized to fatty acid methyl esters (FAME) by mild alkaline methanolysis. The organic phase was evaporated to dryness under gentle nitrogen stream and reconstituted with an N-hexane solution with internal standard (C 23:00). PLFAs were analyzed through a Shimadzu 2010 Gas Chromatograph equipped with a flame ionization detector (GC-FID) using a fused siloxane capillary column (BPX-70; 30 × 25 mm i.d.; film thickness 0.25 μm SGE, Inc., Austin, TX). Individual PLFAs were identified by comparison of retention times with commercially available standard mixtures (FAME and BAME mix, Supelco) and quantified by correlating the peak areas to that of the internal standard. Results were given as relative percentage abundance.