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Microbial communities of polluted sub-surface marine sediments

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ABSTRACT

Microbial communities of coastal marine sediment play a key role in degradation of petroleum contaminants. Here the bacterial and archaeal communities of sub-surface sediments (5–10 cm) of the chronically polluted Priolo Bay (eastern coast of Sicily, Italy), contaminated mainly by n-alkanes and biodegraded/weathered oils, were characterized by cultural and molecular approaches. 16S-PCR-DGGE analysis at six stations, revealed that bacterial communities are highly divergent and display lower phylogenetic diversity than the surface sediment; sub-surface communities respond to oil supplementation in microcosms with a significant reduction in biodiversity and a shift in composition; they retain high biodegradation capacities and host hydrocarbon (HC) degraders that were isolated and identified. HC-degrading Alfa, Gamma and Epsilon proteobacteria together with Clostridia and Archaea are a common feature of sub-surface communities. These assemblages show similarities with that of subsurface petroleum reservoirs also characterized by the presence of biodegraded and weathered oils where anaerobic or microaerophilic syntrophic HC metabolism has been proposed.

1. Introduction

Coastal environments, especially the semi-enclosed basins characterized by low turnover of seawater such as the Mediterranean Sea, are exposed to high levels of contamination by petroleum hydrocarbons due to the intense maritime traffic and high presence of sources of pollution along their entire coastline (Bargiela et al., 2015; Duran et al., 2015; Daffonchio et al., 2013). Petroleum hydrocarbons (HC) are classified as persistent pollutants which remain trapped in the sediments for long periods of time making them a permanent pollution source (McGenity et al., 2012) which have adverse effects on human and environmental health (Varjani, 2017). A part of petroleum HC is degraded in seawater in relatively short time (days or months) while, when the oil reaches the coast, it has a longer residence time in the marine environment (Hazen et al., 2016). The oil contamination can prevent ventilation of upper layers resulting in the development of extensive anoxic zones. Bacterial communities of coastal sediments play a key role in the degradation of these contaminants under aerobic, microaerophilic and anaerobic conditions making bioremediation a concrete alternative to physical and chemical methods for minimizing coastal pollution (Xue et al., 2015). Understanding diversity, composition, and biodegradation ability of microbial communities that inhabit contaminated sediment is the starting point for bioremediation actions. Coastal sediments are heterogeneous habitats and show a high dissimilarity in the composition of bacterial communities among different sites (Acosta-González and Marqués, 2016); much research in recent years has contributed to increasing the knowledge on diversity of indigenous microbial populations of contaminated sediment in order to exploit their metabolic potential (Daffonchio et al., 2013; Bargiela et al., 2015). Most studies have investigated the diversity of HC degrading microbial communities colonizing superficial sediment of polluted sites in the Mediterranean Sea (Amer et al., 2015; Catania et al., 2015; Jeanbille et al., 2016). Sediments generally have a higher microbial abundance and diversity within the marine habitat and bacterial HC degrading communities in marine coastal environments reveal functional differences and different potential for the biodegradation of pollutants; each site is populated by different bacterial communities, generally dominated by Proteobacteria. Among the dominant taxa, specialized hydrocarbonoclastic bacteria were identified in most chronically polluted sites (Catania et al., 2015; Varjani, 2017). However, contamination levels and the differential bacterial distribution of sub-surface sediment is still poorly known. Most studies analyzing the effect of hydrocarbon pollution on subtidal sediments have generally analyzed the uppermost centimeters and in some cases the depth of

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sampled material has not even been detailed (Bargiela et al., 2015, Acosta-González and Marqués, 2016). The bacterial communities inhabiting anaerobic sediments are poorly known and their response to oiling merits particular attention (Miralles et al., 2007). The aim of this study was to explore the diversity and biodegradation potential of microbial communities in sub-surface marine sediments (5–10 cm below the sediment surface) of the Priolo Bay (eastern coast of Sicily, Italy, Central Mediterranean). The Priolo Bay is a Site of Community Importance (SCI) situated in proximity to the Augusta Harbour, affected for decades by pollution from industrial and petrochemical plants (Di Leonardo et al., 2014). The bioremediation potential of superficial sediment and seawater of the Priolo Bay was evaluated previously (Catania et al., 2015) and the most polluted superficial sediments (0–5 cm) were found to host higher bacterial diversity, and higher abundance and diversity of cultivable HC degraders than less polluted stations. Oil and oil-derived hydrocarbon degrading bacteria were isolated from superficial sediment and assigned to *Alcanivorax*, *Marinobacter*, *Halomonas* and *Vibrio* (Catania et al., 2015). In this work, the microbial communities of sub- surface sediments from 5 to 10 cm depth from the Priolo Bay were characterized by DGGE (Denaturing Gradient Gel Electrophoresis) fingerprinting analysis immediately after sampling and after enrichment in oil supplemented microcosms. The degrading capacity of sub-surface sediment microbial communities was analyzed by GC-FID analysis and isolation of HC degraders was carried out under aerobic and anaerobic conditions. This study provides the characterization of the bioremediation potential and describes microbial communities involved in degrading the hydrocarbons in rarely studied subsurface sediments of a chronically polluted area.

2. Materials and methods

2.1. The study area

Priolo Bay is a coastal marine basin located along the South-East coast of Sicily (Italy, Central Mediterranean Sea) in the southern part of Augusta Bay; the northern sector of Augusta Bay includes the heavily-industrialized Augusta Harbour. The areas of Augusta and Priolo have been declared “areas of high risk of environmental crisis” due to the high levels of contamination, and the Priolo Bay was included among the Sites of National Interest (SIN). Moreover the Priolo Bay is part of the Saline di Priolo, a Site of Community Importance (SIC-ZPS ITA090013, Habitats Directive 92/43/EEC) characterized by a coastal wetland affected by water brackish subject to temporary summer drying.

Granulometric data in the Augusta Bay show a settling mainly pelitic and sandy pelitic almost everywhere, probably influenced by rocky outcrops and by the particular structure of the substrate in the area that determines the presence of more coarser and heterogeneous sediments in the band coastal area and close to the dams (ICRAM, 2008).

The Bay of Augusta is subject to pollution by petrochemical plants and wastewater treatment plants, dense urbanization and intense tanker traffic transporting crude and refined oil to and from the refinery located at this site. The high concentrations of Hg, HCB and HC ($C > 12$), identified within the Augusta Bay, are superior to any environmental quality standard defined at national and international level (ICRAM, 2008; Signa et al., 2017).

The southern sector of Augusta Harbour is in connection to the Priolo Bay by the Scirocco inlet (300 m wide,13 m deep) and the Priolo Bay is influenced by the release of contaminants from the Augusta Harbour being in direct connection with it (Di Leonardo et al., 2014). Total PAH, n-alkanes and trace element (mainly As, Cd, Hg, Ni) analysis performed on the superficial sediments of Priolo Bay revealed an irregular spatial distribution of contaminants with sediments close to the Augusta harbour recording greater contaminant concentrations (Di Leonardo et al., 2014; Catania et al., 2015; Signa et al., 2017).

Sedimentation rate was previously calculated in Priolo Bay through ^{210}Pb data (Di Leonardo et al., 2017). Nevertheless, analyses carried out in cores collected from bare sediment, indicated highly disturbed sedimentation, probably related to sediment reworking, and did not allow any estimation of sedimentation rate. The only data available is from a core collected in dead matte of *Posidonia oceanica*, where sediment accumulation rate was 1.2 mm yr^{-1} over the last 120 yr. A much higher sediment accumulation rate was measured by Bellucci et al., 2012 in the Augusta Bay, but this could be influenced by massive sediment dredging activities within the harbour and illegal dumping and discharge offshore (Bellucci et al., 2012).

2.2. Sediment collection

The sediment samples were collected at the Priolo Bay, on the South-East coast of Sicily (Italy, Central Mediterranean Sea), in July 2012. Sediment was sampled through sterile Plexiglas cores (20 cm long, 5 cm diameter) as described in Catania et al. (2015), from three stations located along a northern transect (N1: N 37° 9′ 58,3″, E 15° 12′ 2.6″- depth 5 m; N2: N 37° 10′ 3,7″, E 15° 12′ 21.7″-depth 10 m; N3: N 37′ 10′ 9.3″, E 15° 12′ 53,8″- depth 20 m) and three stations located along a southern transect (S4: N 37° 9′ 20.5″, E-15° 13′ 1.3″-depth 5 m; S5: N 37° 9′ 30.6″, E 15° 13′ 9.8″-depth 10 m; S6: N 37° 9′ 46.8″, E 15° 13′ 20.6″ - depth 20 m; Fig. 1). The samples were transported to the laboratory in a cool box and used for immediate chemical and microbiological analysis and DNA extraction. Aliquots from the layer 5–10 cm were stored at −80° and at −20 °C under glycerol (20% final concentration) for further analysis.

Grain size analysis was carried out on bulk sediment pre-treated with hydrogen peroxide and Na-Hexametaphosphate to remove organic matter and avoid particle flocculation. Then it was wet-sieved through a 63 μm net to separate mud from coarser particles, which were electromechanically dry-sieved through a DIN ISO 3310-1 standard sieve set to obtain a grain size series with intervals of 1/2 Φ ($\Phi = -\log 2$ Ø mm). The pH of sediment samples was measured using a pH meter and probe. The probe was calibrated using the standard solutions (pH 4, 7, and 10 buffers) and was submerged directly into the sample.

2.3. Analysis of hydrocarbons

Total hydrocarbons were extracted from sub-surface sediment following the 3550C EPA (Environmental Protection Agency) procedure. Briefly, a mixture of CH_2Cl_2:CH_3COCH_3 (1:1, v/v) was added to sediment samples. The mixture was sonicated for 30 min in ultrasound bath (Branson 1200 Ultrasonic Cleaner, Branson, USA). Samples were further shaken at 150 × *g* for 30 min, centrifuged for 10 min at 5000 × *g* and supernatant was passed through a ceramic column filled with sodium sulphate 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 to 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 split less 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 sub-samples for each station were analyzed.

2.4. Set-up of microcosms and biodegradation of HC

Microcosms were set up in an anaerobic chamber to prevent exposure to the atmospheric oxygen, by transferring of 0.5 g of each subsurface sediment in 10 ml glass serum bottle containing 5 ml mineral medium ONR7a (Teknova, CA, USA) supplemented with 1% (v/v) of