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Abundance and diversity of sedimentary bacterial communities in a coastal productive setting in the Western Irish Sea

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ABSTRACT

The bacterial community composition and biomass abundance from a depositional mud belt in the western Irish Sea and regional sands were investigated by phospholipid ester-linked fatty acid profiling, denaturing gradient gel electrophoresis and barcoded pyrosequencing of 16S rRNA genes. The study area varied by water depth (12–111 m), organic carbon content (0.09–1.57% TOC), grain size, hydrographic regime (well-mixed vs. stratified), and water column phytodetrital input (represented by algal polyunsaturated PLFA). The relative abundance of bacterial-derived PLFA (sum of methyl-branched, cyclopropyl and odd-carbon number PLFA) was positively correlated with fine-grained sediment, and was highest in the depositional mud belt. A strong association between bacterial biomass and eukaryote primary production was suggested based on observed positive correlations with total nitrogen and algal polyunsaturated fatty acids. In addition, 16S rRNA genes affiliated to the classes Clostridia and Flavobacteria represented a major proportion of total 16S rRNA gene sequences. This suggests that benthic bacterial communities are also important degraders of phytodetrital organic matter and closely coupled to water column productivity in the western Irish Sea.

1. Introduction

Primary production in the marine photic zone accounts for ~44 to 50 Gt C a^{-1} (Harvey, 2006). As a result of the exponential decrease in particulate organic matter with water depth, whereby less than 1% remains at water depths of 4000 m (Suess, 1980), sediments on continental margins account for about 90% of total sedimentary organic matter and are an important component of the global carbon cycle (Hedges and Keil, 1995). Prokaryotes, despite their cell size, are estimated to represent between 15% and 30% of total living biomass (Kallmeyer et al., 2012), and play vital roles in marine ecosystems and in global organic matter cycling (Gooday, 2002; Lochte and Turley, 1988). Bacteria comprise the majority of prokaryotes in global shallow photic waters (Herndl et al., 2005; Karner et al., 2001) and in surface sediments (Biddle et al., 2008; Lipp et al., 2008) and are key players in sedimentary organic matter mineralization (Gooday et al., 1990; Jørgensen, 1982; Lovley and Phillips, 1986).

To date, research on the marine sedimentary microbial diversity, composition and function has largely concentrated on specific environments such as cold seeps, gas hydrate zones, deep-sea sediments and hydrothermal vents (e.g. Boetius et al., 2000; Inagaki et al., 2006; Lloyd et al., 2006; Roalkvam et al., 2012; Teske et al., 2002). Reports from surface marine sediments on continental shelves indicate that the most abundant phylotypes are often the Gamma- and Deltaproteobacteria, the Bacteroidetes (in particular Flavobacteria), Firmicutes (often Clostridia), and the Planctomycetales (Bissett et al., 2006; Bowman et al., 2003; Hunter et al., 2006; Llobet-Brossa et al., 1998; Musat et al., 2006; Ravenschlag et al., 2001). However, despite the importance of coastal and shelf zones in the global carbon cycle, and the recognized key role microbes play in biogeochemical cycling, there remains a relative paucity of data on the composition, abundance and functions of sedimentary microbial communities in coastal settings (Gomes et al., 2013; Hunter et al., 2006; Paisse et al., 2008; Teske et al., 2011; Zhang et al., 2008).

The Irish Sea (Fig. 1) represents a productive, dynamic, and ecologically and commercially important setting (Gowen and

Abbreviations: BFA, Bacterial fatty acids; BRFA, Branched fatty acids; DGGE, Denaturing gradient gel electrophoresis; MUFA, Monounsaturated fatty acids; OUT, Operational taxonomic unit; PUFA, Polyunsaturated fatty acids; PLFA, Phospholipid ester-linked fatty acids; SFA, Saturated fatty acids; SRB, Sulfate-reducing bacteria

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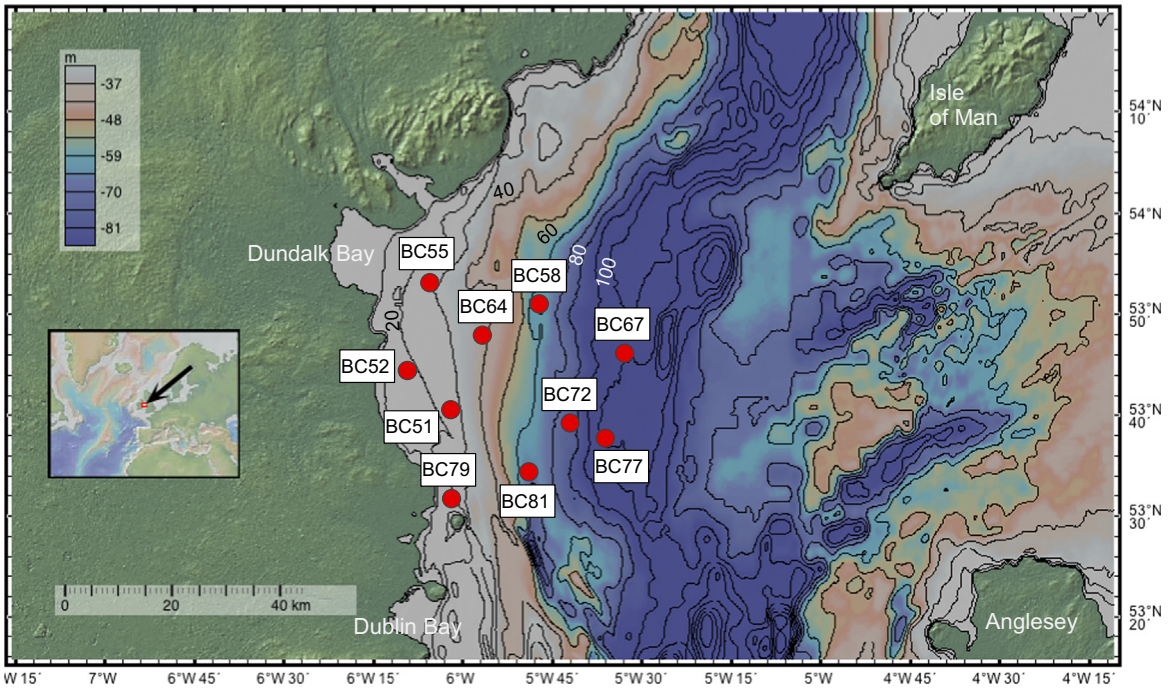

Fig. 1. Bathymetric map showing the location of surface sediment samples described in this study.

Stewart, 2005), which is understudied in terms of prokaryote diversity and ecology (Carty and Litchfield, 1978; El Hag and Fogg, 1986; Floodgate et al., 1981, 1986; Litchfield and Floodgate, 1975; Turley and Lochte, 1986). It is characterized by large regional differences in hydrographic and sedimentological conditions, nutrient chemistry and ecology (Gowen and Stewart, 2005; Hill et al., 1994; Horsburgh et al., 2000). In the western Irish Sea, the formation of an annual seasonal gyre dominates the circulation of the region during late spring and summer and separates the surrounding well-mixed areas by tidal mixing fronts (Hill et al., 1994; Horsburgh et al., 2000). This seasonal gyre and the deeper waters in the northwest region (north of 53.5°N) is characterized by weaker hydrodynamic conditions, allowing the formation of an extensive mud belt. In contrast, the sedimentary environment in the coastal and southern regions (south of 53.5°N) is dominated by sand (O'Reilly et al., 2014a, 2014b). In this study we assessed the abundance and composition of bacterial communities from surface sediments between the mud belt and regional sands by analysis of phospholipid ester-linked fatty acids (PLFA) derived from bacterial biomass (White and Ringelberg, 1998), combined with denaturing gradient gel electrophoresis (DGGE) and barcoded pyrosequencing of 16S rRNA genes.

2. Materials and methods

2.1. Sampling and bulk physical/chemical analysis

The ten surface sediments in this study were sampled in the western Irish Sea (Fig. 1) in June 2010 as part of INFOMAR (INtegrated Mapping FOr the sustainable develoment of Ireland's MArine Resources) survey CV10_28. Samples were taken using a Reineck Boxcorer aboard the RV Celtic Voyager (Fig. 1). Sediment pushcores were taken and stored at −20 °C onboard and in the laboratory. Particle size analysis, TOC and TN were performed as previously described (O'Reilly et al., 2014a). Sediment redox (E_h) was measured using an ORP ProcessProbe Ag/AgCl redox probe (Bradley James Corp., Bedford, UK).

2.2. Lipid biomarker analysis

Lipids were extracted and analyzed according to previously described methods (O'Reilly et al., 2012, 2014a). In brief, freeze-dried and powdered surface (0–2 cm) sediment samples ($n=10$) were extracted using a modified Bligh–Dyer method (White and Ringelberg, 1998). Total lipid extracts were desulfurized with activated copper, and fractionated into neutral, glycolipid and polar lipid fractions by solid phase extraction as previously described (O'Reilly et al., 2014a). Phospholipids in the polar fraction were derivatized with 0.5 M sodium methoxide (50 °C, 30 min). PLFA monounsaturation position was confirmed by formation of dimethyl disulfide adducts as outlined by Nichols et al. (1986). For details of GC–MS analysis parameters and quantification, see O'Reilly et al. (2012, 2014a).

2.3. DNA extraction and 16S rRNA gene DGGE

DNA was extracted from sediment samples ($n=10$) using the POWERSOIL DNA isolation kit (MO BIO, Carlsbad, US). PCR reactions (50 µL) were carried out using a DNA Engine DYAD Peltier Thermal Cycler. DGGE primers 2 and 3 were used to amplify the variable V3 region of the bacterial 16S rRNA gene as outlined by Muyzer et al. (1993). A touch-down PCR was performed as follows: denaturation step of 95 °C for 5 min; followed by 20 cycles of 95 °C for 1 min, 60 °C for 1 min and 72 °C for 3 min, with a 0.5 °C decrease per cycle for the annealing step. This was followed by 8 cycles of 95 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min. DGGE was performed using the CBS Scientific DGGE 2401 system with denaturing gradients from 20% to 80% (80% denaturant consisted of 5.6 M urea and 32% v/v formamide). Electrophoresis was performed at constant voltage and temperature of 90 V and 60 °C respectively, for 16 h. The gels were then stained using 1× SYBRGold nucleic acid stain (Invitrogen, Paisley, UK) for 45 min and imaged by transillumination.

2.4. 16S rRNA gene barcoded pyrosequencing

Barcoded 16S rRNA gene pyrosequencing was carried out on five samples according to Berry et al. (2011). Samples from sites