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Using community metabolomics as a new approach to discriminate marine microbial particulate organic matter in the western English Channel

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ABSTRACT

Metabolomics provides an unbiased assessment of a wide range of metabolites and is an emerging 'omics technique in the marine sciences. We use 'non-targeted' community metabolomics to determine patterns in metabolite profiles associated with particulate organic matter (POM) at four locations from two long-term monitoring stations (L4 and E1) in the western English Channel. The polar metabolite fractions were measured using ultra-high performance liquid chromatography Fourier transform ion cyclotron resonance mass spectrometry (UHPLC-FT-ICR-MS), and the lipid fractions by direct infusion Fourier transform ion cyclotron resonance mass spectrometry (DI-FT-ICR-MS); these were then analysed to statistically compare the metabolite distributions. Results show significantly different profiles of metabolites across the four locations with the largest differences for both the polar and lipid fractions found between the two stations relative to the smaller differences associated with depth. We putatively annotate the most discriminant metabolites revealing a range of amino-acid derivatives, diacylglyceryltrimethylhomoserine (DGTS) lipids, oxidised fatty acids (oxylipins), glycosylated compounds, oligohexoses, phospholipids, triacylglycerides (TAGs) and oxidised TAGs. The majority of the polar metabolites were most abundant in the surface waters at L4 and least abundant in the deep waters at E1 (E1-70m). In contrast, the oxidised TAGs were more abundant at E1 and most abundant at E1-70m. The differentiated metabolites are discussed in relation to the health of the phytoplankton as indicated by nutrients, carbon and chlorophyll, and to the dominance (determined from metatranscript data) of the picoeukaryote *Ostreococcus*. Our results show proof of concept for community metabolomics in discriminating and characterising polar and lipid metabolite patterns associated with marine POM.

1. Introduction

Particulate organic matter (POM) in the ocean plays a crucial role in global carbon cycling in terms of the turnover of organic metabolites, driving the biological pump and the generation of climatically active gases. The composition of marine POM is largely determined by microbes, principally the carbon fixing phytoplankton. Fixed phytoplankton carbon and other elements are incorporated into a wide range of organic compounds or metabolites which are then acted on by biotic factors including interactions between bacteria, viruses and zooplankton, resulting in recycling and remineralisation of POM. In addition to biotic factors, a diverse range of abiotic interactions such as light, temperature and salinity also affect POM composition.

Lipids, carbohydrates and amino acids are the primary groups of metabolites that make up the fundamental building blocks of

Abbreviations: ANOVA, analysis of variance; Chl-a, chlorophyll-a; CID, collision-induced dissociation; DAG, diacylglyceride; DGTS, diacylglyceryltrimethyl homoserine; DI, direct infusion; FT-ICR, Fourier transform ion cyclotron resonance; GC-MS, gas chromatography–mass spectrometry; IRMPD, infrared multiphoton dissociation; JCVI, J. Craig Venter Institute; LV, latent variable; MS, mass spectrometry or mass spectrometric; OVOCs, oxygenated volatile organic compounds; PCA, principal component analysis; PEG, polyethylene glycol; PLS-DA, partial least squares discriminant analysis; POM, particulate organic matter; PUFA, polyunsaturated fatty acids; QC, quality control (sample); RP, reversed-phase; RSLC®, rapid separation liquid chromatography; UHPLC, ultra-high performance liquid chromatography; TAG, triacylglyceride; WEC, western English Channel.

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microbes in the oceans. These primary metabolites and other groups of secondary metabolites, especially pigments, have often been used as organic biomarkers to investigate the source, composition and degradation of marine POM especially its alteration down through the water column and into the sediment (e.g. Handa and Tominaga, 1969; Wakeham and Lee, 1989; Lee et al., 2004; Rontani et al., 2011). As a sub-set of the lipids, the fatty-acids are key nutrients affecting physiological performance, and have been used as organic biomarkers to assess trophic transfer and food quality (e.g. Kainz et al., 2004). Pigments, central to light harvesting in photosynthesis, have been used widely to provide chemotaxonomic characterisation of phytoplankton in a wide range of contrasting oceans (see review by Jeffrey et al., 1997). Pigments together with pigment degradation products and particulate carbon have also been used to track the fate of POM down the water column (Bidigare et al., 1986; Llewellyn and Mantoura, 1996). Overall though, a lack of biochemical techniques has hindered the full chemical identification of POM and a significant proportion remains uncharacterised (Lee et al., 2004). Recent advancements in analytical and computational tools are now enabling a revolution in the investigation of microbial communities and their interactions with the environment (Larsen et al., 2012).

Advancements in mass spectrometry (MS), hyphenated technologies and associated software have enabled the development of the newest of the 'omic techniques, metabolomics. Metabolomics involves the non-targeted unbiased analysis of large suites of low molecular weight organic molecules or metabolites (typically 50–1500 Da) and combined with statistical analysis enables the discovery of relationships between metabolites, organism physiology and the environment. Metabolomics complements genomics, transcriptomics and proteomics and represents an important addition to the 'omics toolkit especially because it provides the closest molecular link to phenotype (Vemuri et al., 2005). This unbiased analysis of organic matter contrasts to the more traditional targeted analysis of predefined compound groups, the latter remaining important for the testing of specific hypotheses. As the polarity of molecules within organic material is highly diverse, the extraction and analysis of all metabolites using one method cannot be achieved. Therefore extraction and analysis in metabolomics is generally divided into that required for the polar or hydrophilic metabolite fraction and that required for non-polar or lipophilic metabolite fraction, often termed lipidomics.

Metabolomics has already demonstrated its important role in several research fields, including bioenergy, environmental interactions, functional genomics and gene discovery, secondary metabolism, genome-wide association mapping, and metabolic modelling in higher organisms and microbial systems (Tang, 2011). It has also been used to study environmental stress responses in plants (reviewed in Arbona et al., 2013). Metabolomics has also been applied in studies of individual strains of microalgae, e.g., on the model algae *Chlamydomonas* (Lee and Fiehn, 2008; May et al., 2008), the cyanobactera *Synechococcus* and *Synechocystis* (Baran et al., 2010; Schwarz et al., 2013) and on the diatom *Skeletonema marinoi* (Vidoudez and Pohnert, 2011). Notably non-targeted metabolomics has revealed a number of unexpected metabolites in *Synechococcus* sp. PCC 7002, such as histidine betaine (hercynine), its derivatives and several unusual oligosaccharides including a range of oligohexoses (Baran et al., 2010). The potential of combining metabolomics and genomics for the identification of novel biosynthetic genes was recently highlighted in a study on a diverse range of cyanobacteria (Baran et al., 2013). Metabolomics has also revealed that shifts from high to low CO_2 levels induce a coordinated change in the central C/N-metabolism in *Synechocystis* 68034 (Schwarz et al., 2011).

Metabolomics, when applied to whole systems or communities direct from the environment, is termed community or meta-metabolomics, akin to metagenomics. An example of where community metabolomics is being used widely is in determining the effects of gut microflora on human health (Nicholson et al., 2012; Turnbaugh and Gordon, 2008). It was also used recently in a soil ecology study to assess the entire microbial community of a soil sample to determine how it responds to factors such as pollution and climate change (Jones et al., 2014). There have been few community metabolomics studies in aquatic or terrestrial environments to date and it has not yet been used to study natural populations of marine microbes.

The temperate marine ecosystem of the western English Channel (WEC) provides an excellent platform to assess the metabolite compositions of the POM in an un-biased manner and to provide proof of concept for marine community metabolomics. Monitoring in the WEC has been occurring for over forty years making it one of the best studied marine regions in the world. The two main monitoring stations, L4 and E1, are seasonally stratified from late April until September and both have a spring and autumn phytoplankton bloom. Long-term monitoring of phytoplankton using microscopy counts at L4 over a period of 15 years has revealed a consistent pattern of bloom formation with diatoms reaching maximum abundance in mid-April followed by peaks in abundance of *Phaeocystis* and coccolithorphorids (Widdicombe et al., 2010). Phyto-flagellates numerically dominate throughout the year gradually increasing in spring with maximum abundance towards late May (Widdicombe et al., 2010). Overall the biological community in the WEC is variable, shifting over the annual cycle in response to abiotic factors such as seasonal fluctuations in light and nutrients, turbulence, temperature and other meteorology factors such as wind and cloud (Widdicombe et al., 2010; Smyth et al., 2010).

As part of the monitoring at these stations an extensive database has been compiled providing information on the phytoplankton and zooplankton community populations. Additional routine measurements at these stations include irradiance, salinity, temperature, chlorophyll, nutrients, carbon and nitrogen, phytoplankton and zooplankton counts, and photosynthetic pigments (www.weco.uk). In terms of metabolite analysis, targeted analysis of pigments using HPLC has been undertaken in the WEC for over ten years although correlating pigments with phytoplankton carbon and particulate carbon remains a challenge (Llewellyn et al., 2005). Short term, targeted metabolite studies at L4 have focussed on fatty acids to determine zooplankton fecundity (Pond et al., 1996). Additionally a group of UV sunscreen metabolites, mycosporine-like amino acids, have been studied at L4 showing temporal variation according to phytoplankton composition and solar irradiance (Llewellyn and Harbour, 2003). Recently, preliminary metagenome and metatranscriptome analyses have been used to characterise the microbial populations at L4 revealing a robust seasonal structure for the bacterial community (Gilbert et al., 2010a,b).

Here we build on our long term understanding of the western English Channel describing the first preliminary community metabolomics study to chemically characterise the POM in the WEC. Our study is focused on the >0.7 μm to <200 μm fraction of POM primarily composed of phytoplankton. Our investigation was enhanced by collecting samples in collaboration with JCVI (J. Craig Venter Institute) in May 2009, whose aim was to molecularly and genetically characterize the microbes in the WEC. There were four main aims to our study; 1. To evaluate community metabolomics as a new state-of-the- art approach to statistically discriminate different microbial populations in the WEC; 2. To putatively annotate abundant lipid and polar metabolites to determine trends across the sampling locations; 3. To compare