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Diatom derived dissolved organic matter as a driver of bacterial productivity: The role of nutrient limitation

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ABSTRACT

Variable inorganic nutrient concentrations were used to generate silicate (Si) or nitrogen (N) limited conditions in cultures of the marine diatom *Skeletonema costatum*. Dissolved organic matter (DOM) harvested in the nutrient limited phase of these cultures was added to a natural bacterial community. Enhanced bacterial abundance and bacterial production were observed, in comparison to un-supplemented controls, when Si-limited (Si-DOM) rather than N-limited (N-DOM) diatom derived DOM or inorganic nutrients was added. This indicates that the bacterial population was limited by organic rather than inorganic resources but only Si-DOM had sufficient lability to alleviate this. Within the bacterial assemblage, a notable increase in the proportion of γ-*Proteobacteria* was evident on receipt of only Si-DOM. Assessment of the composition of the added DOM suggested that the observed dynamics were related to organic matter composition rather than molecular size as, within the Si- and N-DOM, the proportions of low and high molecular weight compounds were similar, but the polysaccharide and protein signatures were different.

1. Introduction

Dissolved organic matter (DOM) is an important substrate for bacterial growth in aquatic ecosystems (Azam et al., 1994). DOM is mainly produced by phytoplankton (Carlson and Hansell, 2002), but may also result from riverine (Hedges et al., 1997) or atmospheric (Buatmenard et al., 1989) inputs, anthropogenic sources (Jickells, 1998), micro- and meso-zooplankton grazing (Strom et al., 1997) or viral lysis (Proctor and Fuhrman, 1992).

Diatoms (Bacillariophyceae) are a particularly important component of the phytoplankton, contributing 20–25% of the global net marine primary production (Werner, 1977). The magnitude of the spring diatom bloom in coastal waters suggests that factors governing the quantity and quality of the DOM produced in the nutrient limited senescent phase of this event are likely to be of critical importance to the subsequent abundance, composition, and productivity of the bacterial communities.

Nutrient stress has been shown to stimulate DOM excretion by phytoplankton (Maranon et al., 2005). Moreover, as such stress influences the chemical composition of phytoplankton (Obernosterer and Herndl, 1995), it may influence the quality and quantity of DOM released (Puddu et al., 2003). For diatoms, Myklestad (1974, 1995) demonstrated that glucans (polymers of glucose) are common storage products, which provide a suite of bioavailable compounds (Hama et al., 2004). Glucans only accumulate in diatoms when growth is suboptimal (Myklestad, 1974), and are influenced by the form of nutrient limitation experienced by the population (Gilpin et al., 2004). For example, enhanced glucan concentrations are found under N-limitation. Under silicate limiting conditions, however, silicification processes within diatom cells are compromised leading to weaker cell walls and potentially "leaky" cells (Martin-Jézéquel et al., 2000), with the potential for enhanced DOM release. Hence, the form of nutrient limitation is likely to influence both the quantity and quality of the diatom produced DOM and, we hypothesise, the resultant productivity and composition of the bacterial populations utilising this organic matter.

In Scottish and other temperate coastal waters, *Skeletonema* sp. are commonly the dominant spring bloom organisms (Tett, 1992). Hence, the period of their bloom cessation and decline might be expected to introduce significant DOM loading. The inorganic N:Si ratio within these waters is often close to one (Fehling et al., 2006) with both nutrients having the potential to be the limiting factor that arrests the spring diatom bloom. Our study, therefore, investigated the effect of N and Si-limitation on the release of DOM by the important diatom *Skeletonema costatum* and the influence of this material on the abundance, productivity and composition of a natural bacterial assemblage collected from coastal waters.

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2. Methods

The experiments were conducted in two stages. Initially, cultures of *S. costatum* were grown to a post exponential phase under either N or Si-limitation, with DOM produced collected by ultrafiltration.

In stage 2, this harvested DOM was added to freshly collected samples of natural marine bacteria. The subsequent changes in bacterial abundance, production and community composition that occurred in response to the added DOM were measured.

To quantify and characterise the DOM harvested in stage 1 for addition in stage 2, the produced DOM material was analysed by HTCO (high temperature catalytic oxidation) and HPLC (high-performance liquid chromatography).

2.1. Stage 1: S. costatum cultures: generation and characterisation of DOM

Axenic cultures of *S. costatum*, previously isolated from Loch Creran, Scotland (CCAP, 1077/9), were grown in 10 l carboys containing autoclaved filtered seawater (8 l) with a slightly modified f/2 medium, with EDTA excluded to avoid an additional source of dissolved organic carbon (DOC). Cells were maintained at 15 °C under a 12/12 light/dark photo-cycle (cool-white daylight spectrum tubes) and a photon flux density of 160 μmol m^{-2} s^{-1}. All equipment were both acid washed and steam-sterilised (121 ° C; 15 min) prior to use. All experiments were conducted in duplicate. After inoculation the culture vessels remained closed systems with samples being removed by siphon into sterile syringes. All sampling was conducted in a class 2 laminar flow cabinet. Lack of bacterial contamination was confirmed by fluorescence microscope enumeration of DAPI stained cells, as described below.

To generate DOM from N and Si-limited phytoplankton, different initial concentrations of inorganic nitrate and silicate were used. For N-limitation, a N:Si ratio of 1:3 (40 μM and 120 μM, respectively) was used, whereas for Si-limitation, the N:Si ratio used was 3:1 (120 μM and 40 μM, respectively). Phosphorus and other trace elements were added in excess.

To determine when the stationary phase had been reached, samples for chlorophyll a (chl *a*) determination were collected daily from the culture vessels. The samples were filtered onto 25 mm glass fibre filters (type A/E, Pall Corporation) and stored frozen prior to analysis by fluorometry after extraction with 90% acetone. To identify the point at which nutrient limitation (N or Si) had been reached, parallel determinations for dissolved nitrate, nitrite and ammonium (DIN), silicate (Si) and phosphate (P) were measured using a Lachat Quickchem 8000 FI nutrient analyzer (Hach Lange, Colorado, USA).

2.1.1. Biomass determination

S. costatum cells were viewed using an inverted light microscope (Axiovert 200, Zeiss) mounted with a digital camera (Axiocam, Zeiss) coupled to an image analysis software (Axiovision, Zeiss). Cell volume was determined from measurements of cell length and width (Davidson et al., 2002). Live cell volume was obtained using a conversion factor of 1.33 from measurements made on Lugol fixed cells (Montagnes et al., 1994). The algorithms of Montagnes et al. (1994) and Menden-Deuer and Lessard (2000) were used to estimate carbon biomass from volume, which were found to give identical results.

2.1.2. DOM harvest, ultrafiltration, quantification and characterisation

DOM from the *S. costatum* cultures was harvested during the nutrient limited stationary phase (after 14 days growth in both nutrient regimes) by gravity filtration using pre-ashed GF/F filters (Whatman 0.4 μm nominal porosity) to remove particulate material and transparent exopolymers (TEP). The resultant filtrate, pooled from duplicated cultures and referred to as the DOM fraction, was sub-divided for further processing and analytical determinations, as well as for use in the stage 2 experiments (see below).

A volume of the collected DOM was subjected to tangential filtration (Schleicher & Schuell) using a 1 kDa membrane. DOC and dissolved organic nitrogen (DON) concentrations in the low molecular weight (LMW <1 kDa) and high molecular weight (HMW >1 kDa) fractions were determined following treatment with 85% orthophosphoric acid of 10 ml samples. Samples were flame sealed in precombusted glass ampules prior to analysis by HTCO with coupled infrared and chemiluminescence detection (Alvarez-Salgado and Miller, 1998).

Polysaccharides were extracted from the DOM fraction by adding two volumes of cold ethanol and extensively dialyzing the precipitated material with deionised water (ELGA Ultrapure system, Veolia Water, UK) prior to freeze drying. The polysaccharide extracts were analysed by high-performance liquid chromatography using an Agilent 1100 chromatograph (Agilent Technologies) equipped with a refractometer and diode-array UV detector. A PL gel filtration chromatography column (Polymer Laboratories; 7.5×300 mm) was used at 30 °C. The mobile phase was 0.1 M $NaNO_3$ with a flow rate 0.6 ml min^{-1}. Dextran standards of M_r range 50–1400 kDa (Sigma) were used for molecular weight estimation.

The polysaccharide extracts were analysed for total carbohydrate content following the method of Dubois et al. (1956) with glucose as standard. Total protein concentrations were determined using the BCA protein assay kit (Sigma, St. Louis, MO) with bovine serum albumin as the standard. Assays were performed on 1 to 4 mg ml^{-1} solutions of the polysaccharide extracts derived from Si- and N-limited phytoplankton.

2.2. Stage 2: DOM addition experiments

Seawater was collected from 10 m with a Niskin bottle from the Scottish west coast fjord, Loch Creran (station C5, 56° 32.05′ N, 05° 19.40′ W), then pre-screened with a 160-μm mesh net to remove macro-zooplankton, and the sample stored in a Nalgene carboy during its rapid (<2 h) return to the laboratory. Water was further screened by gravity filtration through GF/F filters to reduce the nanoflagellate density using our previous protocols (Lønborg et al., 2009a, 2010).

100 ml of the DOM fraction generated from both N- and Si-limited *S. costatum* cultures in stage 1 was added (separately) to duplicate 1 l bottles containing 9 parts of the natural bacterial community. Inorganic nutrients as nitrate, silicate and phosphate were added with the DOM to prevent inadvertent inorganic limitation (10 μM, 10 μM, and 1 μM, respectively) of bacterial growth. To provide control comparisons, two additional treatments received inorganic nutrients only (no DOM) or no nutrient addition. In the following section, treatments are referred to as (1) "Si-DOM"—in receipt of DOM from Si-limited phytoplankton, (2) "N-DOM"—in receipt of DOM from N-limited phytoplankton, (3) "Inorganic"—in receipt of inorganic nutrients alone and (4) "control"—no additions made. All experimental and sampling equipment were acid cleaned and rinsed with deionised water to prevent any organic C or N contamination. Flasks were incubated at 15 °C for 72 h and sampled every 24 h, after gentle shaking, for bacterial abundance (BA), bacterial production (BP), and bacterial community composition. Contamination by heterotrophic (HNF) and phototrophic (PNAN) nanoflagellates was assessed at the same frequency. Inorganic and organic nutrient concentrations were determined at t = 0 and t = 48.

2.3. Enumeration of bacterial abundance, production, community composition, and heterotrophic, phototrophic and nanoflagellate abundance

BP was determined from [*methyl*-^{3}H] thymidine incorporation following the protocol outlined in Davidson et al. (2007). Carbon equivalents were calculated using 30.2 fg C $cell^{-1}$ determined for