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Metal binding properties of the EPS produced by *Halomonas* sp. TG39 and its potential in enhancing trace element bioavailability to eukaryotic phytoplankton

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Abstract An emergent property of exopolysaccharides (EPS) produced by marine bacteria is their net negative charge, predominantly conferred by their high uronic acids content. Here, we investigated the EPS produced by an algal-associated marine bacterium, *Halomonas* sp. strain TG39, for its capacity to sequester trace metals and mediate their bioavailability to eukaryotic phytoplankton. Metal analysis of the purified EPS revealed that it contained high levels of K, Ca, Mg and several essential trace metals, including Zn, Cu, Fe and the metalloid Si. Desorption experiments with marine sediment showed that the EPS possessed a specific binding capacity for Ca, Si, Fe, Mn, Mg and Al. Depending on the ionic conditions, Fe was the third or fourth most highly-adsorbed metal out of 27 elements analyzed. Experiments employing Fe-limited synthetic ocean seawater showed that growth of the marine diatom *Thalassiosira weissflogii* (axenic strain) was enhanced when incubated in the presence of either purified EPS or EPS that had been pre-exposed to marine sediment, compared to non-EPS amended controls. This growth enhancement was attributed to the EPS binding and increasing the bioavailability of key trace metal elements, such as Fe(III). Since the bacterium used in this study was originally isolated from a marine micro-alga, this work highlights the possibility that bacterial associates of eukaryotic algae could be influencing the bioavailability of Fe(III) to phytoplankton via their production of polyanionic EPS. More widely, this work reinforces the potential importance of marine bacterial EPS in trace metal biogeochemical cycling.

Keywords Exopolysaccharide (EPS) · Iron · Metal ions · Eukaryotic phytoplankton · Marine bacteria

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Introduction

The world's oceans contain a total dissolved organic carbon content that is comparable in mass to the carbon in atmospheric CO_2 (Hansell and Carlson 1998). Much of this dissolved organic matter (DOM)

exists as biopolymers (ca. 10–25 % of total oceanic DOM) that undergo reversible transition between colloidal and dissolved phases (Chin et al. 1998; Verdugo 1994). A major source of this material derives from the synthesis and release of exopolysaccharides (EPS) by bacteria and phytoplankton. EPS can serve a variety of functions, including biofilm formation, the solubilisation of hydrophobic organic chemicals, and in the binding and fate of cationic species (Decho 1990; Santschi et al. 1998). A key property of many chemically characterized marine bacterial EPS is that they have a net negative charge, attributable to any number of anionic groups (e.g. COO^-, $C–O^-$, SO_4^-). Compared to EPS produced by marine eukaryotic phytoplankton (Bhaskar and Bhosle 2005) and non-marine bacteria (Ford et al. 1991), marine bacterial EPS characteristically contains higher levels of uronic acids, notably D-glucuronic and D-galacturonic acid (Kennedy and Sutherland 1987). Hence, the polyanionic nature of marine bacterial EPS has been a topic of growing interest in recent years, in particular the need to characterize its role in marine biogeochemical processes (Bhaskar and Bhosle 2005).

A number of reports have described marine bacterial EPS binding heavy and toxic metal ions such as Cd, Cr, Pb, Ni, Cu and Al (Beech and Cheung 1995; Bhaskar and Bhosle 2006; Gutierrez et al. 2008; Iyer et al. 2005; Schlekat et al. 1998). The rationale to many of these studies was commercial, and few have thoroughly addressed the ecological implications of EPS-metal complexes in marine systems. In a study by Ford et al. (1987), the EPS produced by bacteria involved in corrosion processes were found to selectively complex different metals, and the authors implicated this as being important in the corrosion process. Two studies reporting on the heavy metal-binding capacity of EPS produced by hydrothermal vent bacteria proposed that this might represent a survival strategy by reducing their exposure to toxic metals released from the hydrothermal vents (Loaec et al. 1997, 1998). Elements such as Na, Mg, Ca, K, Sr and Si, which are major constituents of seawater, have been shown to be adsorbed by marine bacterial EPS (Gutierrez et al. 2008).

Evidence is now indicating that polyanionic EPS may have a role in controlling Fe(III) bioavailability. Recent studies that have shown single anionic residues, such as glucuronic and galacturonic acids (Hassler et al. 2011b; Hassler and Schoemann 2009), and purified marine bacterial EPS containing high levels of uronic acids (Gutierrez et al. 2008; Hassler et al. 2011a), effectively bind Fe(III) and can promote the uptake of this trace metal by eukaryotic phytoplankton (Hassler et al. 2011b). The implications of this are significant because of the abundance of EPS and transparent exopolymer in the ocean (Verdugo et al. 2004) and because Fe(III) is an essential trace metal that limits primary production in up to 40 % of the open ocean (Boyd et al. 2007; Martin et al. 1994).

In the present study, EPS isolated from a marine bacterium, itself originally isolated from a eukaryotic phytoplankton culture, was examined with respect to its capacity to bind metals and affect the growth of a marine micro-alga. The EPS, produced by *Halomonas* sp. TG39, has been characterized as a polydisperse and high molecular mass polyanionic glycoprotein (Gutierrez et al. 2007, 2009). Halomonads are frequently linked with the production of large quantities of EPS rich in uronic acids (Arias et al. 2003; Bejar et al. 1998; Calvo et al. 1998, 2002; Quesada et al. 1993), and this raised questions regarding their role in binding metals. In order to identify the metals that *Halomonas* sp. TG39 EPS may complex, a previously characterized sediment matrix was used as the source of a defined suite of up to 27 metal species. To examine how this polymer might in turn affect metal bioavailability to eukaryotic phytoplankton, *Thalassiosira weissflogii* was challenged with EPS that had been primed with or without marine sediment, and its growth monitored.

Materials and methods

Organisms and media

The marine bacterium *Halomonas* sp. TG39 produces EPS that was characterized previously (Gutierrez et al. 2007, 2009). This strain was originally isolated from a laboratory culture of an unclassified Chrysophyte (strain CCAP958/1) that was obtained from the River Crouch estuary in Essex, UK. Selection of this bacterial strain was based on its growth on solid synthetic seawater medium amended with *n*-hexadecane as the sole source of carbon and energy. Strain TG39 was grown in marine broth (ZM/1) composed of ¾-strength naturally aged seawater, peptone (0.5 %), yeast extract (0.1 %), and supplemented after

autoclaving with filter-sterile (0.2 μm) trace elements and vitamins to final concentrations as previously described (Blackburn et al. 1989). For EPS production, ZM/1 amended with glucose (ZM/1 + Glc) was prepared by adding a pre-autoclaved glucose solution to ZM/1 to a final concentration of 1 % (w/v).

An axenic laboratory culture of the marine diatom *Thalassiosira weissflogii* CCMP1010 (National Center for Marine Algae and Microbiota, Maine, USA) was used in this study. The strain was maintained in f/2 + Si medium and in a temperature-controlled illuminated incubator. Experiments with this strain were performed using an Fe-depleted synthetic ocean water amended with silicate (SOW + Si) (Sunda et al. 2005). All chemicals used in the preparation of SOW + Si medium were of analytical grade or higher, and all glassware used in its preparation had been washed with 10 % HCl.

Purification of the EPS

For production and purification of the EPS, strain TG39 was grown in 2-l Erlenmeyer flasks containing 800 ml of ZM/1 + Glc. After incubating for 3 days (150 rpm; 28 °C), the cells were removed by cross-flow filtration (0.2 μm; Schleicher & Schuell, Dassel, Germany). The resultant cell-free spent medium was diluted with at least two volumes of distilled water as previous work had shown this improved on the recovery of the EPS during passage through a 100 kDa cut-off membrane cassette (Schleicher and Schuell) (Gutierrez et al. 2009). The retentate fraction, containing the concentrated EPS, was dialysed against 5 l of distilled water and then precipitated with 7 % (w/v) KCl and 3–4 volumes of cold 99 % ethanol. The precipitated EPS was harvested, dialysed against 20 l of distilled water, and then lyophilized. The distilled water used in the extraction of the EPS was of 18 MΩ/cm quality.

Trace metal desorption experiments

The ability of the EPS to complex 25 transition metals and two metalloids (Si and As)—hereon, collectively referred to as metals—from a marine sediment sample was performed under conditions of low (10 mM KNO_3) and high (0.6 M NaCl) ionic strength in order to evaluate the effect of salts on metal desorption. The latter condition simulated the ionic strength of seawater. The sediment sample used in these experiments was obtained from Garroch Head, a site located in the river Clyde near Glasgow (Scotland). The sediment was air-dried and ground to a fine powder. Selected characteristics of the sediment were as follows: dry bulk density, 0.45 ± 0.12 g/cm^3; porosity, 0.80 ± 0.07; salt content, 6.57 ± 2.04 %; organic carbon, 4.25 ± 1.03 %; organic nitrogen, 0.43 ± 0.11 %; elemental C/N ratio, 11.28 ± 1.48.

To characterize the metal species that the EPS could complex, a series of 15-ml polypropylene vials were prepared containing 0.5 g of sediment to which 5 ml of the EPS (5 mg/ml) dissolved in either 10 mM KNO_3 (low ionic-strength condition) or 0.6 M NaCl (seawater ionic-strength condition) was added. The mixtures were then incubated in the dark overnight with shaking (150 rpm; 21 °C). The vials were centrifuged (13,000×*g* for 10 min) and the supernatant from each vial removed and stored at −20 °C for subsequent metal analysis. Metal analysis on the supernatant fractions was performed by inductively coupled plasma-atomic emission spectrometry (ICP-AES) and ICP-mass spectrometry (ICP-MS), as described previously (Gutierrez et al. 2008; Howe et al. 2007; Swan et al. 2003). The metal concentration of the purified EPS (not challenged with sediment) was also analyzed. All treatments were conducted in triplicate for each experiment (i.e. in 10 mM KNO_3 or 0.6 M NaCl). Subsamples of the supernatant fractions from the treatments performed using 10 mM KNO_3 were stored at −20 °C for subsequent addition to *Thalassiosira weissflogii* CCMP1010 cultures (see below). These samples were labelled as Treatment A for supernatant fractions derived from triplicate incubations with EPS at 5 mg/ml, and Treatment B for supernatant fractions derived from triplicate incubations of the sediment without any added EPS (Table 1).

Trace metal bioavailability experiments

To investigate the effect of the purified EPS and the supernatant fractions (Treatments A and B) on the growth of *T. weissflogii*, four sets of three 125-ml Erlenmeyer flasks were prepared, each containing 30 ml of SOW + Si and 1.4 ml of the respective treatment, as detailed in Table 1. The final concentration of EPS in flasks amended with Treatments A and D was ca. 0.4 μM, based on an estimated molecular

Table 1 Treatment regime used for growth of *Thalassiosira weissflogii*

Treatment	1.4-ml amendment to cultures of *T. weissflogii*
A	EPS in KNO_3 conditioned with sediment
B	KNO_3—(no EPS) conditioned with sediment
C	KNO_3 only
D	EPS dissolved in KNO_3 only

weight for this polymer of ca. 600 kDa (Gutierrez et al. 2009). Flasks amended with Treatment C (KNO_3 only) acted as the "no EPS" control. Each of the 12 flasks was then inoculated with exponentially-growing *T. weissflogii* cells to concentrations of 10.5–10.8 ln (cells ml^{-1}). Prior to inoculation the cells had been washed five times with sterile SOW medium in order to eliminate carry-over of trace metal ions. The final nitrate concentration in each flask equated to 5.7×10^{-4} M. The flasks were incubated at 16 °C with 12:12 light/dark cycle and at a photon flux density of 25 μmol s^{-1} m^{-2}. Algal cell numbers were routinely monitored using a haemocytometer. All glassware used in these experiments had been acid-washed (10 % HCl) prior to use.

Background concentrations of Fe in SOW + Si were measured via the method of Biller and Bruland (2012). The seawater was pre-concentrated in an offline manifold using the chelating resin Nobias-chelate PA1 (Hitachi High-Technologies), eluted with 1 N nitric acid, and analyzed on a magnetic sector ICP-MS (Biller and Bruland 2012).

Statistical analysis

The Student's *t* test was used to test for any significant differences between the treatments at the 99, 95 and 90 % confidence intervals.

Results and discussion

Trace metal composition associated with the EPS

Of the 27 metal ions that were analyzed (Table 2), seven were found to be intrinsically-associated with the purified EPS at concentrations ≥0.001 % of EPS dry weight: K (0.81 %), Ca (0.03 %), Si (0.02 %), Mg (0.01 %), Zn (0.01 %), Cu (0.005 %) and Fe (0.001 %). The latter four are recognized as essential trace metals for eukaryotic phytoplankton, and Si, a metalloid, is essential to the growth of diatoms (Boyd et al. 2007; Brand et al. 1983; Brzezinski et al. 2003; Coale 1991; Coale et al. 2003; Peers et al. 2005; Ragueneau et al. 2000). Based on this analysis it is not possible to infer whether this intrinsic metal specificity is biologically meaningful. However, Fe is the 16th most abundant metal in seawater at a concentration of ca. 2.0 μg/l, which is ≥5 orders of magnitude lower than other metal ions such as Na (1.0×10^7 μg/l), Mg (1.3×10^6 μg/l) and Ca (4.1×10^5 μg/l) (Gerlach 1981). Yet here, Fe was the 7th most abundant metal associated with the EPS at 13.4 μg/g EPS (Table 2). Since extensive dialysis (7–14 kDa cut-off) of the EPS against 18 MΩ/cm quality water should have removed non-complexed metals, the Fe concentration found associated with the purified EPS is likely to be due to the coordinated complexation of this metal to the carboxyl moieties of uronic acids (Hassler et al. 2011b). Low concentrations of several other essential (Ni, Mo, V, Co) and heavy/toxic (Pb, Cr, Cd, U, As) trace metals were also found associated with the EPS (Table 2).

Only two types of marine bacterial EPS have previously been investigated for their capacity to bind metals, both produced by *Pseudoalteromonas* species—strains TG12 (Gutierrez et al. 2008) and CAM036 (Hassler et al. 2011a; Mancuso Nichols et al. 2004). The EPS produced by strain TG12 was found to have an uronic acid content of 28.7 % and of 13 metals analyzed, Ca (141,500 μg/g EPS), Sr (2700 μg/g EPS) and Fe (140 μg/g EPS) were found to be the most abundant metals associated with this polymer (Gutierrez et al. 2008). The EPS produced by strain CAM036 also contained a similar level of uronic acids (25 %), and of 12 metals analyzed, the highest levels of metal bound to the EPS were Fe (70.6 μg/g EPS), Cu (35.8 μg/g EPS), Zn (25.5 μg/g EPS) and Al (4.1 μg/g EPS) (Hassler et al. 2011a). The apparent theme shared by these polymers is their high levels of uronic acids and predominance of iron as a major associated metal. The binding of Fe and other essential trace metals by uronic acids (Gyurcsik and Nagy 2000; Hassler et al. 2011a) and polymers enriched with these acidic sugars has been reported (Schoemann et al. 2001; Sreeram et al. 2004). An important ecological role of these acidic groups was recently demonstrated by Hassler et al. (2011b) who showed that uronic acids

Table 2 Profile of metals intrinsically bound to the *Halomonas* EPS, and ability of the EPS to desorb sediment-adsorbed metals under conditions of low (10 mM KNO_3) and seawater (0.6 M NaCl) ionic strengths

Metal	EPS-associated[a] (μg/g EPS)	Metals desorbed (μg/g EPS)[b]	
		10 mM KNO_3	0.6 M NaCl
Ca	253.3 ± 64.3	5566.7 ± 152.7	506.7 ± 58.6
Si	173.3 ± 23.1	2466.7 ± 115.5	133.3 ± 5.8
Fe	13.4 ± 2.3	888.5 ± 11.0	123.1 ± 2.0
Mn	BDL	192.2 ± 3.4	6.8 ± 0.3
Mg	100.0 ± 0.0	183.3 ± 18.6	420.0 ± 81.9
Al	<15.0[c]	24.7 ± 4.0	106.0 ± 1.3
K	8113.3 ± 41.6	BDL	BDL
Zn	69.2 ± 3.8	BDL	BDL
Cu	51.7 ± 2.0	BDL	9.4 ± 1.0
Ba	0.6 ± 0.3	BDL	BDL
Li	<15.0[c]	BDL	1.1 ± 1.0
Sr	<1.0[c]	BDL	4.8 ± 0.9
Be	0.1 ± 0.0	BDL	BDL
V	0.3 ± 0.0	3.8 ± 0.5	2.1 ± 0.6
Cr	1.5 ± 0.0	7.2 ± 0.1	4.1 ± 0.6
Co	0.2 ± 0.0	0.7 ± 0.0	1.1 ± 0.1
Ni	2.8 ± 0.0	1.4 ± 0.1	BDL
As	0.1 ± 0.0	3.8 ± 0.1	1.6 ± 0.5
Rb	3.9 ± 0.0	BDL	BDL
Mo	1.4 ± 0.0	BDL	0.1 ± 0.1
Cd	0.4 ± 0.1	0.5 ± 0.0	0.2 ± 0.1
Cs	0.1 ± 0.0	BDL	BDL
Pb	7.6 ± 1.0	BDL	BDL
Th	0.4 ± 0.0	0.1 ± 0.0	BDL
U	0.4 ± 0.0	1.3 ± 0.0	BDL
Ti	<1.0[c]	BDL	3.4 ± 0.1
Na	BDL	BDL	ND

BDL below detection limit, *ND* not determined

[a] Non-dialyzable metal concentration associated with the lyophilized EPS prior to sediment exposure

[b] EPS dissolved in and exposed to sediment in 10 mM KNO_3 or 0.6 M NaCl. Values represent the amount of metal desorbed by the EPS after subtracting background metal concentrations contributed by the EPS and sediment to the aqueous phase

[c] Values are the limit of determination

can enhance the bioavailability of Fe(III) to eukaryotic phytoplankton of the Southern Ocean.

Effect of EPS on desorption of metals from marine sediment

Analysis of the metal species desorbed from sediment by EPS showed that Ca, Si, Fe and Mg were found to be significantly desorbed ($P < 0.01$) at concentrations >100 μg of metal per gram of EPS in both ionic strength treatments (Table 2). Mn was desorbed at these levels only in the 10 mM KNO_3 treatment, whereas the same was observed for Al in only the 0.6 M NaCl treatment. The ionic concentration of the aqueous phase had a significant ($P < 0.01$) effect on the amount of each metal species that was desorbed. Under the low ionic strength condition (10 mM KNO_3), Ca, Si, Fe and Mn were desorbed at higher levels, whereas Mg and Al were desorbed at higher levels in seawater ionic strength (0.6 M NaCl).

Notably, Fe was the third (889 μg/g EPS) and fourth (123 μg/g EPS) most highly desorbed metal in the KNO_3 and NaCl treatments, respectively (Table 2). The negative effect of increasing ionic strength on the ability of bacterial polymers to bind metals (e.g. Ca, Si and Fe) has been observed previously (Bhaskar and Bhosle 2006; Lores and Pennock 1998). Of the remaining 21 metals detected, all were desorbed from the sediment at <10.0 μg per gram of EPS in both ionic strength treatments. No desorption of K, Zn, Ba, Be, Rb, Cs, Pb and Na was detected in either treatment, although Na could not be quantified in the 0.6 M NaCl treatment due to its high concentration (Table 2).

Of the six metals preferentially desorbed by the EPS, Ca, Si, Mg and Fe were also the predominant metals found intrinsically associated with the purified polymer. This indicates that the EPS has a specific affinity for these metals. It can be hypothesized that this selectivity may serve an ecological role for the producing bacterium. Firstly, the EPS is able to sequester essential trace metals, most notably Fe, which may then be readily accessible to high affinity uptake systems, such as siderophores or cell surface ferric transporters (Amin et al. 2012). Secondly, the EPS may serve to block cellular toxicity by locking up heavy metals, such as Cd and Cr. A priori, the EPS may, via the same mechanisms, inadvertently benefit other organisms living in the same niche, such as micro-algal cells.

Effect of EPS on the bioavailability of trace metal nutrients to *T. weissflogii*

To test the hypothesis that the EPS produced by *Halomonas* sp. TG39 could affect growth of eukaryotic phytoplankton, we examined the growth of the marine diatom *T. weissflogii* (axenic strain CCMP1010) when incubated in the presence and absence of the purified EPS, or with the two supernatant fractions (Treatments A and B) that were derived from the desorption experiments conducted with marine sediment in KNO_3 (Table 1). Since concentrations for many of the metals were higher in these KNO_3 supernatant fractions, compared to those derived from NaCl treatments, they were selected based on the assumption that they would amplify the effect of EPS-bound metals on the growth of the diatom.

In order to identify the metal species that could influence the growth of *T. weissflogii* in each of the different treatments, the final concentrations of the 6 major, 6 essential trace and 6 toxic metals present in the SOW + Si medium in the four treatments were calculated (Table 3). As shown in Table 3, the essential trace metals Zn, Mn, Co, Mo, Cu and metalloid Si were all present in Treatment A at concentrations that were equal or higher to that in the other three treatments. The Fe concentration was, however, ca. 10-fold higher in Treatment A (131 nM) compared to that in the other three treatments (15.5–17.2 nM). Concentrations of the toxic metals Al, As, Cr, Pb, U and Cd varied from almost no difference to 100-fold between the treatments. Treatments A and B, which had been pre-exposed to sediment, had very similar levels of these toxic metals, whereas the non-sediment exposed Treatments C and D had lower levels of these metals.

The mean growth rates (0.22–0.28 day^{-1}) of *T. weissflogii* were not significantly different ($P > 0.20$) between any of the four treatments during the exponential phase of growth (days 0–7) (Fig. 1). However, differences between all four treatments began to emerge after day 7. Compared to the untreated control containing KNO_3 only (Treatment C), the sediment conditioned EPS (Treatment A) produced elevated growth yields ($P = 0.12$) reaching average cell concentrations 1.8-fold higher between days 10–13. By contrast, cells grown in sediment conditioned KNO_3 (Treatment B) produced cell concentrations that averaged 1.8-fold lower than the untreated control (Treatment C) during the same time period ($P = 0.2$). Although Treatment A contained higher concentrations of the toxic metals Pb, U and Cd, their potential negative impact on growth was either mitigated by the EPS, plausibly by the EPS locking up these metals and preventing cellular toxicity, or countered by the presence of a ca. 10-fold higher concentration of Fe (131 nM) as derived from supplementation of this treatment with EPS-desorbed sediment metals. This Fe was potentially bioavailable because it was likely to be present as Fe(III) via coordinated chemistry of uronic acid moieties of the *Halomonas* EPS. In comparison to Treatments A–C, growth of *T. weissflogii* with the unconditioned EPS (Treatment D) achieved significantly ($P < 0.01$) higher levels of growth, reaching cell concentrations that averaged 4.5-fold higher compared to the untreated control Treatment C (Fig. 1). Even by end of the experiment, growth of the Treatment D cells

Table 3 Final concentration of metals in SOW + Si medium of treatments A, B, C and D

	Final metal concentration			
	Treatment A	Treatment B	Treatment C[a]	Treatment D
Major metals (M):				
Na	4.81×10^{-1}	4.81×10^{-1}	4.81×10^{-1}	4.81×10^{-1}
K	1.03×10^{-2}	1.03×10^{-2}	1.02×10^{-2}	1.02×10^{-2}
Mg	5.46×10^{-2}	5.46×10^{-2}	5.46×10^{-2}	5.46×10^{-2}
Ca	1.05×10^{-2}	1.05×10^{-2}	1.05×10^{-2}	1.05×10^{-2}
Sr	6.38×10^{-5}	6.38×10^{-5}	6.38×10^{-5}	6.38×10^{-5}
Si	1.01×10^{-4}	1.00×10^{-4}	1.00×10^{-4}	1.00×10^{-4}
Essential trace metals (nM):				
Fe[b]	131.0	15.6	15.5	17.2
Zn	99.3	80.8	79.7	87.1
Mn	211.0	187.0	121.0	121.0
Co	50.1	50.4	50.3	50.3
Mo	105.0	105.0	100.0	100.0
Cu[c]	31.6	20.6	19.6	25.3
Toxic metals (nM):				
Al	39.7	27.1	0.0	2.3
As	1.9	1.5	0.0	0.01
Cr	1.7	3.2	0.0	2.1
Pb	0.5	0.0	0.0	0.3
U	0.06	0.004	0.0	0.01
Cd	0.2	0.06	0.0	0.04

All of the treatments contained selenium (a non-metal), as Na_2SeO_3, at 1.0×10^{-8} M final concentration. Standard deviations were <10 %

[a] Concentrations of all the metals in Treatment C (non-supplemented control) reflect those found in SOW medium

[b] Iron concentrations were measured as per the method of Biller and Bruland (2012)

[c] Copper can also be included in the group of toxic metals because at high enough concentrations it can cause toxicity effects for some species of phytoplankton (Levy et al. 2007)

was continuing, albeit at a decreasing rate. In terms of the total Fe concentration, Treatment D was very similar to Treatments B and C, yet the growth of *T. weissflogii* was significantly better in D, which had been supplemented with EPS, compared to in B and C that did not contain EPS. Growth in Treatment A was also better than in B and C, the former of which had also received EPS.

The growth results suggest that the presence of EPS (either unconditioned or sediment conditioned) in Treatments A and D was the main factor that enabled *T. weissflogii* to reach the higher growth yields observed. This apparent growth enhancement could be due to several factors. First, the presence of uronic acids—ca. 30.8 % of total monosaccharide content (Gutierrez et al. 2007)—were responsible for coordinating the complexation of Fe(III) via their carboxyl moieties, and increasing the biological availability of this metal ion to *T. weissflogii*. This is corroborated by recent reports (Hassler et al. 2011a, b; Hassler and Schoemann 2009) showing that saccharides such as galacturonic acid, glucuronic acid, dextran, alginic acid and bacterial EPS specifically increase the bioavailability of Fe to field and laboratory phytoplankton. Furthermore, the EPS could also affect the solubility and bioavailability of other essential trace metal species such as Cu, Co, Mn and Zn that can limit algal growth either because of an excess or limitation (Brand et al. 1983; Coale 1991; Levy et al. 2007; Morel et al. 1991; Scharek et al. 1997). The higher growth yield of *T. weissflogii* in the presence of unconditioned EPS (Treatment D) compared to sediment conditioned EPS (Treatment A) was unexpected because the latter treatment contained higher levels of Fe (131.0 nM) which should have stimulated growth. However, this may be explained by the higher concentrations of toxic metals present in Treatment A which had markedly countered the growth-stimulating properties of the Fe.

Ecological implications

Our results add to a body of data showing that bacterial EPS can complex a diverse suite of metals, including a number of essential trace metals (e.g. Fe, Zn, Mn, Si)

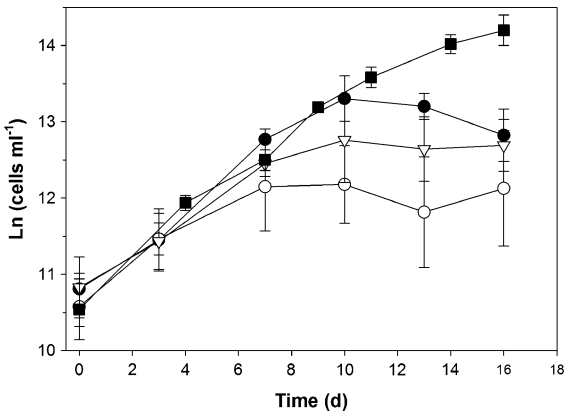

Fig. 1 Effect of metals desorbed from sediment by the *Halomonas* EPS on the growth of *Thalassiosira weissflogii* CCMP1010 in synthetic ocean seawater. Incubations supplemented with supernatant fractions: Treatment A (sediment conditioned EPS in 10 mM KNO_3, *filled circle*); Treatment B (sediment conditioned 10 mM KNO_3 alone, *white circle*); Treatment C (10 mM KNO_3 only, *white down-pointing triangle*); Treatment D (EPS dissolved in 10 mM KNO_3 only, *filled square*)

and toxic metal species (e.g. Cd, U and Pb). Furthermore, we were able to show that the *Halomonas* EPS was capable of mediating a significant enhancement to the growth of *T. weissflogii*. The mechanism underlying metal complexation and growth enhancement is believed to be related to the high levels of uronic acids present in this and other EPS macromolecules (Gutierrez et al. 2007; Mancuso Nichols et al. 2004) that function to increase the solubility and bioavailability of Fe and/or other essential trace elements, or reducing the exposure of algal cells to toxic metals. Overall, this data suggests that bacterial EPS could have an important role in biogeochemical cycling of trace metals in the marine environment.

The question arises as to what function does marine bacterial EPS have in terms of biogeochemical processes and primary production? EPS-producing bacteria are recognized to produce these macromolecules for biofilm formation, colonisation and attachment to particulates (Decho 1990; van Boekel 1992). How significant the bacterial contribution to the pool of acidic polysaccharides is remains poorly understood, although Santschi et al. (2003) have suggested that this contribution is quantifiable based on data showing a correlation between bacterial productivity and acidic polysaccharide concentration. Another possibility is that the production of EPS is part of a mutualistic relationship with eukaryotic phytoplankton, analogous to the proposed siderophore-mediated iron-for-carbon mutualism (Amin et al. 2009). The observation that saccharides resulted in phytoplankton taking up ≥2-fold more Fe than the bacterioplankton (Hassler et al. 2011b) certainly implies there could be a greater benefit to the alga than to the EPS-producing bacterium. Clearly, further work is required to explore the relationship between the chemical structure of EPS produced by other marine bacterial species and their capacity to bind metals, especially Fe, and to quantify the contribution bacteria make to the acidic polysaccharide pool of DOM.

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