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Metabolic characterization of a model heterotrophic bacterium capable of significant chemical alteration of marine dissolved organic matter

Byron Pedler Sherwood [a,*,1,2], Emily A. Shaffer [a,3], Kalani Reyes [a,4], Krista Longnecker [b], Lihini I. Aluwihare [c], Farooq Azam [a]

[a] Marine Biology Research Division, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093, USA
[b] Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA
[c] Geosciences Research Division, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093, USA

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ABSTRACT

The marine bacterium *Alteromonas* sp. AltSIO was previously found to consume an equivalent magnitude of surface coastal marine dissolved organic carbon (DOC) as diverse bacterial assemblages (Pedler et al., 2014). In this study, we sought to investigate the potential of AltSIO to alter the chemical composition of marine DOC by characterizing its capacity to metabolize a broad suite of environmentally relevant model substrates. Results showed that AltSIO had a particularly broad capacity to degrade carbohydrates relative to other marine bacteria characterized as generalist heterotrophs. Growth in seawater incubations amended with model neutral sugars and radiolabeled substrates showed that AltSIO preferentially utilized D-galactose and disaccharides, but showed little to no biomass incorporation or respiration of D-glucose. Lastly, analysis of ambient dissolved organic matter (DOM) from time-course mesocosms by ultrahigh resolution mass spectrometry showed that both AltSIO grown in pure culture and a mixed bacterial community significantly altered ambient DOM, yet the alteration appeared uniform across chemical classes for both treatments. This study provides insight into the physiological mechanisms of a globally distributed generalist bacterial taxon that has the capacity to significantly alter the geochemistry of marine DOM.

1. Introduction

The dynamic nature of the upper ocean dissolved organic carbon (DOC) cycle is reflected in oceanic seasonal profiles of DOC concentration (Carlson et al., 1994), the radiocarbon signature of surface ocean DOC compared to deep ocean values (Druffel et al., 1992), and a wealth of studies over the past several decades demonstrating significant bacterial growth, enzyme activity, and the transition of microbial community composition over seasonal timescales and following phytoplankton blooms (Carlson, 2002; Carlson and Hansell, 2015). Incubation studies coupling DOC drawdown to increases in bacterial number also confirm the presence of bioavailable DOC in a variety of surface ocean locations (Carlson and Ducklow, 1996; Kirchman et al., 1991; Letscher et al., 2013; Pedler et al., 2014).

The labile DOC pool encompasses the single greatest flux of carbon, up to ~25 Pg $C \cdot y^{-1}$, through the DOM reservoir in the global ocean (Hansell, 2013). Although a variety of compounds participate in upper ocean DOC turnover, evidence supports the important role of dissolved carbohydrates as a conduit of carbon and energy transfer in these ecosystems (Aluwihare et al., 1997; Benner et al., 1992; Ittekkot et al., 1981; Pakulski and Benner, 1994; Repeta and Aluwihare, 2006). Depending on the method of analysis, carbohydrates have been found to comprise between a few percent up to ~30% of total marine DOC in the surface ocean (Benner, 2002; Pakulski and Benner, 1994), and accumulate primarily in the form of polysaccharides (Benner et al., 1992). DOC has been shown to become enriched in dissolved combined neutral sugars (DCNS) following phytoplankton blooms in the field (Borsheim et al., 1999; Ittekkot et al., 1981) and in culture (Aluwihare and Repeta, 1999; Biersmith and Benner, 1998), and can comprise fucose, rhamnose, arabinose, galactose, glucose, mannose, and xylose (Aluwihare et al., 1997; Borch and Kirchmann, 1997; Goldberg et al., 2009; McCarthy et al., 1996). Incubation experiments demonstrate that individual monosaccharides encompass a bioavailable component of the DOC pool exhibiting a range of turnover rates from days to months (Amon et al., 2001; Cowie and Hedges, 1994; Goldberg et al.,

* Corresponding author.
E-mail address: bpedler@gmail.com (B. Pedler Sherwood).
[1] Present address: Department of Oceanography, School of Ocean and Earth Science and Technology, University of Hawaii, Honolulu, HI 96822, USA.
[2] Present address: Daniel K. Inouye Center For Microbial Oceanography: Research and Education, University of Hawaii, Honolulu, HI 96822, USA.
[3] Present address: Department of Tropical Medicine, School of Public Health and Tropical Medicine, Tulane University, New Orleans, LA 70118, USA.
[4] Present address: College of Natural and Applied Science, University of Guam, Mangilao, Guam 96923, USA.

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2011; Kirchman et al., 2001). Yet, few studies have succeeded in connecting changes in the concentration and composition of dissolved carbohydrates to particular bacterial taxa found in the surface ocean (but see Alonso and Pernthaler, 2006; Alonso-Saez and Gasol, 2007; Elifantz et al., 2005).

Nearly half of all newly fixed carbon in the ocean is consumed by marine bacteria daily (Ducklow, 1999; Fuhrman and Azam, 1982), making heterotrophic bacterial activity the primary degradation pathway for labile DOC. Gammaproteobacteria within the family Alteromonadacea have been shown to rapidly respond to labile DOM and account for a significant fraction of active bacterial communities during and after phytoplankton blooms (Tada et al., 2011, 2012). Furthermore, the ecological and geochemical importance of conditionally rare taxa, those typically rare but occasionally prevalent, is becoming better understood (Shade and Gilbert, 2015). For example, in a transect from mesotrophic coastal California waters to the oligotrophic subtropical North Pacific, Dupont et al. (2015) found that alteromonads and pseudoalteromonads comprised a low proportion of metagenomes, but accounted for a significant fraction of global gene transcription. These data provide further evidence for the disproportional contribution of numerically rare taxa to geochemical fluxes and highlight their important role in maintaining ecosystem function (Campbell et al., 2011; Hugoni et al., 2013). Within this context we sought to characterize the metabolic potential of a model taxon shown to employ this ecological strategy, *Alteromonas* sp. AltSIO, a strain with the capacity to contribute as much to DOC drawdown as diverse bacterioplankton consortia (Pedler et al., 2014).

Our goal in this study was to assess the potential impact of AltSIO metabolism on DOC chemical composition by characterizing its physiological capacity to consume an environmentally relevant suite of model compounds. We began by broadly testing the ability of AltSIO to oxidize 95 substrates using BioLog™ plates and compared its metabolic capacity with four additional strains with documented ecological and biogeochemical significance. We then measured the biomass production and DOC consumption of AltSIO grown in seawater amended with 11 neutral sugars, and later quantified the uptake, incorporation, and respiration of three ^{14}C-radiolabeled model sugars. Considering previous findings that this isolate rapidly consumes a significant fraction of coastal DOC, we sought to test the hypothesis that a single bacterial isolate also has the capacity to significantly alter the chemical signature of ambient DOC relative to a native bacterioplankton consortia. We tested this hypothesis by characterizing DOM throughout time-course microcosm experiments using ultrahigh resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS).

2. Methods

2.1. Global distribution of Alteromonas AltSIO 16S rRNA

We obtained 16S rRNA miTAG sequences from all 139 publicly available samples from the TARA Oceans Expedition (Sunagawa et al., 2015) (http://ocean-microbiome.embl.de/companion.html) and compared them to the 16S rRNA sequence of *Alteromonas* sp. AltSIO (accession no. KC758958.1) using LAST (Kielbasa et al., 2011) (http://last.cbrc.jp/). All environmental sequences with 100% sequence identity were counted as hits to *Alteromonas* sp. AltSIO 16S rRNA (Appendix A: Table S1).

2.2. AltSIO genome queries

The genome of *Alteromonas* sp. AltSIO was sequenced by the U.S. Department of Energy Joint Genome Institute (JGI) and is publicly available (NCBI BioProject accession: PRJNA190838). The permanent draft genome was queried using the JGI Integrated Microbial Genomes Expert Review online portal (Markowitz et al., 2009).

2.3. Bacterial isolate-specific single substrate metabolism

The ability to metabolize a suite of 95 individual substrates was tested using BioLog GN2 MicroPlates™ (BioLog, Inc., Hayward, CA) containing single compounds from broad chemical groups including monosaccharides, polysaccharides (di-, tri-, and oligosaccharides), carboxylic acids, organic acids, amino acids, and peptides. Five bacterial isolates were tested and compared including *Alteromonas* sp. AltSIO (Pedler et al., 2014), *Pseudoalteromonas* TW7 (Bidle and Azam, 2001; Bidle et al., 2002), *Vibrio* SWAT-3 (Long and Azam, 2001), Flavobacterium BBFL7 (Bidle and Azam, 2001; Bidle et al., 2002), and *Ruegeria pomeroyi* DSS-3 (Moran et al., 2004). Bacteria were grown in ZoBell medium (5 g peptone, 1 g yeast extract, L^{-1} GF/F filtered seawater) in overnight culture, shaken at 170 rpm at 22 °C then pelleted by centrifugation at 6000 ×*g*, and washed 2× with autoclave-sterilized, GF/F-filtered seawater (AFSW). Cells were resuspended in AFSW to a final cell density of 50% transmittance (~0.30 optical density at 600 nm), equivalent to ~10^8 cells mL^{-1} per manufacturer's recommendation. Cell suspensions (150 μL) of each isolate were added to three separate 96-well microplates and incubated in the dark. Inoculated wells without substrate served as the control. Development of the fluorescent reaction product formazan, an indicator of bacterial respiration, was measured by the absorbance at 590 nm using a microplate reader (Molecular Devises, Sunnyvale, CA). Measurements were made after 2 h of inoculation, then every 24 h for 5 d. Each well was scored as "positive" if the absorbance measured ≥2× the absorbance of the blank (substrate-free, cell inoculated) well within 48 h. Each isolate was scored as "positive" for the ability to metabolize substrate if at least 2 of 3 replicate plates yielded a positive result.

2.4. Alteromonas AltSIO hydrolytic enzyme activity

Ectoenzyme activity of AltSIO was assayed using fluorogenic substrates (Hoppe, 1983; Hoppe et al., 1988) derived from 7-amino-4-methylcoumarin (AMC) and 4-methyl-umbelliferone (MUF) as described (Martinez et al., 1996). Protease activity was measured as the hydrolysis rate of leucine-AMC; α-D-glucosidase was assayed as the hydrolysis rates of MUF α-D-glucoside; β-D-galactosidase was measured as the hydrolysis rate of MUF-β-D-galactoside; and MUF-oleate was used to assay lipase activity. AltSIO was streaked onto a low nutrient (ZoBell diluted 10^3-fold) agar plate from a -80 °C cryogenically preserved glycerol stock, grown for 2 d at 22 °C, then a single colony was used to inoculate a liquid culture for growth in AFSW. After 3 d of growth in AFSW, and while still in exponential growth phase, 3 mL aliquots (in triplicate) were incubated in the dark with 20 μM of each fluorogenic substrate. Blanks consisted of AFSW incubated with each substrate. Solutions of MUF and AMC were used to generate standard curves.

2.5. AltSIO growth response to single sugar amendment in seawater

2.5.1. Experimental setup

The metabolic capacity of AltSIO to utilize specific sugars including disaccharides, monosaccharaides, hexose sugars and pentose sugars was further tested. After first incubating AltSIO for 5.5 d in ambient DOM (0.1 μm filtered seawater) to exhaust the labile DOM fraction, the potential for the addition of specific sugars to stimulate co-metabolism of operationally defined semi-labile DOM was also tested. AltSIO was supplemented with 1 μM ammonium nitrate (NH_4NO_3) and 2 μM of each sugar including sucrose, maltose, mannose, galactose, glucose, fucose, rhamnose, sorbose, fructose, xylose, and arabinose (but note these sugars are later illustrated in total C units). Additional treatments included 2 × 10^4-fold diluted ZoBell media, a DOC re-feed where AltSIO was diluted ~90% and replenished with the original 0.1 μm FSW. Two controls included the addition of i) 1 μM NH_4NO_3 only, and ii) no substrate or NH_4NO_3 addition. A control treatment

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