Metabolic characterization of a model bacterial strain capable of significant chemical alteration of marine dissolved organic matter

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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 shows 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., 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 (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; Tada et al., 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 and colleagues (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 (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 polysaccharides, mono- 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 *Rugeria pomeroyi* DSS-3 (Moran et al., 2004). Bacteria were grown in ZoBell medium (5 g peptone, 1 g yeast extract, L^{-1} seawater) in overnight culture, shaken at 170 rpm at 22^{o}C then pelleted by centrifugation at 6,000xg, and washed 2X 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 600nm), 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 ≥ 2X the absorbance of the blank (substrate-free, cell inoculated) well within 48h. 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^{o}C cryogenically preserved glycerol stock, grown for 2 d at 22^{o}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 x 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 amended with only NH_4NO_3 was included to test for the potential for nitrogen-limited degradation of otherwise labile DOC. Growth response was quantified as total cell abundance and total viable cells determined as colony forming units on ZoBell marine agar plates.

To setup culture incubations, AltSIO was grown from a -80^{o}C glycerol stock as described above, inoculated into AFSW, grown and acclimated for 4 d in the dark at ambient environmental temperature (16^{o}C), transferred to a fresh batch of AFSW, grown for 2 d, then used to inoculate 3.5 L of freshly collected (not autoclaved) 0.1 µm filtered seawater (FSW). Seawater was collected from the Ellen Browning Scripps Memorial Pier ("Scripps pier" hereafter) on 18 March 2014 during an incoming tide, gravity filtered through pre-combusted GF/F filters, then filtered through a pre-flushed 0.1 µm PES filter membrane using a peristaltic pump. AltSIO was grown in 0.1 µm FSW for 5.5 d to allow for the consumption of labile DOM (Pedler et al., 2014), then 200 mL of the culture was aliquoted into 15 1-L pre-combusted glass Erlenmeyer flasks. To each treatment flask, NH_4NO_3 and C substrate was added. Samples for bacterial enumeration by flow cytometry were collected in duplicate for a total of 5 d, fixed with

1% (final conc.) electron microscopy-grade glutaraldehyde (Ted Pella, Inc., Redding, CA) for 20 min at 4 °C, flash frozen in liquid nitrogen, then transferred to -80 °C for storage. Initially, samples were collected at 12 h, 18 h, 24 h, then once per d for 5 d. On d 5, samples were diluted 10^3X and 10^4X and plated onto ZoBell agar to assay total viable cells by enumeration of colony forming units (CFU) (Table S2). Samples for total organic carbon (TOC) analysis were collected at d 0 before and after sugar addition, and at d 5. DOC drawdown of each treatment was calculated by subtracting bacterial biomass carbon from measured TOC concentrations. Biomass C was calculated by multiplying total cell concentration by empirically derived AltSIO-specific carbon content of 63 fg C $cell^{-1}$ (Pedler et al., 2014).

2.5.2. Cell enumeration by flow cytometry

Bacterial cells were enumerated by flow cytometry using a BD Influx™ (Becton Dickinson, San Jose, CA) high-speed cell sorter equipped with a small particle detector. Filter-sterilized (0.2 μm) BioSure® (Grass Valley, CA) sheath fluid was used at 1X concentration with a 70 μm nozzle. Ultra Rainbow Fluorescent Particles (3.8 μm diameter; Spherotech, Warrington, PA) were used to align lasers during instrument setup. Frozen samples were thawed at room temperature, diluted to ~$5x10^5$ cells/mL with filter-sterilize sheath fluid, and stained with 4X (final conc.) SYBR Green I (Life Technologies, Carlsbad, CA) for 30 min in the dark before analysis. Cell fluorescence was excited with a 488 nm argon laser (Sapphire Coherent, Coherent, Santa Clara, CA), and emission was detected using 531/40-Green (FITC) filter. At least 10^5 cells were enumerated per sample.

2.4.3. Cell enumeration by epifluorescence microscopy

Samples were imaged at 1,000X magnification using a Nikon TE2000-U inverted epifluorescence microscope using a CoolSnapHQ CCD camera and analyzed using Nikon NIS-Elements 3.2 software. Cells were enumerated using the nucleic acid stain 4′,6-diamidino-2-phenylindole (DAPI) (Porter and Feig, 1980).

2.4.4. TOC analysis

TOC concentrations were measured by high-temperature combustion using a Shimadzu 500 V-CSN/TNM-1 TOC analyzer as described (Pedler et al., 2014), and calculated from the average of five sample injections (100 μL) from two replicate vials. All samples were analyzed during a single run to limit instrument variability, and interspersed with deep seawater reference material (Hansell, 2005).

2.6. Uptake, incorporation, and respiration of individual sugars

2.6.1 ^{14}C-sugar incorporation into biomass

Incorporation by *Alteromonas* AltSIO of ^{14}C-glucose (Moravek Biochemicals, Inc., Brea, CA), ^{14}C-fructose (Moravek Biochemicals, Inc., Brea, CA), and ^{14}C-galactose (MP Biomedicals, Solon, OH) was tested. AltSIO was grown in AFSW for 2 to 4 d at 16 ^{o}C as described above, then incubated with ~50 nM radiolabeled glucose (255 mCi/mmol), fructose (240 mCi/mmol), and galactose (108 mCi/mmol) for 16 h. Formaldehyde (2% final conc.) was added to "killed controls" prior to substrate addition. After incubation, treatment and control samples were divided into 2 mL aliquots in triplicate, cells were pelleted by centrifugation at 16,000xg at 4 ^{o}C for 10 min, and supernatant was decanted. Cells were washed with 1 mL ice cold AFSW, centrifuged a second time as above, supernatant was decanted, and 1 mL of EcoLite liquid

scintillation cocktail (MP Biomedicals, Solon, OH) was added to each tube. All samples were placed on ice while individual tubes were being processed. From each sample, 100 µL of supernatant was subsampled to quantify total radioactivity in solution. Each sample was counted for ^{14}C disintegrations per min (DPM) using a liquid scintillation counter (PerkinElmer) for 10 min.

2.6.2 Total uptake, biomass incorporation, and $^{14}CO_2$ production of single sugars

To quantify the catabolic and anabolic partitioning of glucose, galactose, and fructose, AltSIO was incubated with 1 µM ^{14}C-labeled substrates for 13-14 h in the dark at 16^{o}C using the method of Karl et al. (1998). AltSIO was inoculated into aged AFSW and grown for 3.5 d (as described above) to a concentration of 1.4 x 10^5 cells/mL, then aliquoted into 27 125-mL borosilicate glass serum bottles (previously 1.2N HCl acid washed and combusted at 450 ^{o}C for 8 h). To each bottle, 1 µM (final conc.) of each substrate was added; 6 bottles per sugar treatment received ^{14}C-labeled substrate, and 3 bottles per sugar treatment received cold substrate. After incubation, 3 replicate bottles per substrate were sacrificed for quantification of ^{14}C incorporation into cell biomass by filtering onto 25 mm GF-75 (0.3 µm nominal pore size), and 3 replicate bottles per substrate were sacrificed for quantification of $^{14}CO_2$ production. Total radioactivity in solution was measured from each bottle immediately after substrate addition (T_0), and prior to processing for ^{14}C biomass and $^{14}CO_2$ (T_{final}). Triplicate cold sugar incubation bottles were subsampled at T_0 and T_{final} to quantify the change in cell abundance.

2.6.3. AltSIO uptake and incorporation of ^{3}H-L-leucine

The uptake and incorporation by *Alteromonas* AltSIO of ^{3}H-L-leucine was tested. AltSIO was grown in AFSW as described for single sugar uptake assays. AltSIO was incubated with a calculated concentration of 1, 3 and 10 nM ^{3}H-L-leucine (144 Ci/mmol) for 1.5 h at 16 ^{o}C. Control samples were placed on ice 15 min prior to substrate addition and remained on ice for the duration of the incubation. All samples were processed in an identical manner to ^{14}C-sugar biomass incorporation experimental procedures.

2.7. Impact of AltSIO on coastal dissolved organic matter composition measured by FT-ICR-MS

2.7.1. Microcosm experimental design

Seawater was collected from the Scripps pier, 0.1 µm filter-sterilized, and used to test growth of various treatments on ambient DOM (see Supplemental Methods). Experimental treatments included i) AltSIO in <0.1 µm seawater (6 replicates); ii) free-living bacterial seawater community (SWC) comprised of 10% GF/F FSW in 90% 0.1 µm seawater (3 replicates); iii) SWC inoculated with AltSIO (3 replicates); and iv) bacteria free <0.1 µm seawater control. All samples were incubated in 20 L polycarbonate carboys. Incubations were sampled for bacterial abundance and TOC daily for 10 d, and on d 40. On d 0, 3, 5, 10, and 40 samples were collected for DOC analysis by FT-ICR-MS (Supplemental Methods).

2.7.2. FT-ICR-MS: DOM processing and analysis

DOM was extracted from acidified (pH 2.5) 0.2 µm-filtered seawater samples (1 L) using 6 mL/1 g Bond Elut PPL solid phase extraction cartridges (Agilent, Santa Clara, CA) as described in Dittmar et al. (2008), and eluted with 100% methanol. Mass spectrometry was performed on DOM extracts by electrospray ionization (ESI) using a 7 Tesla FT-ICR-MS

(Thermo Scientific) located at the Woods Hole Oceanographic Institution as described in Bhatia et al. (2010). Spectra were aligned as described (Kido Soule et al., 2010) to generate a list of *m/z* values from all spectra and compiled into a matrix of 24 samples by 15, 298 peaks with unique atomic masses. Relative peak heights were transformed to presence/absence (peak height = 1/0), and a hierarchical cluster analysis was performed using Bray–Curtis distance measure and Ward's linkage method as described in Koch et al. (2005). Two samples were removed from all analyses because they failed to ionize and produce a comparable number of detectable peaks relative to other samples. Molecular formulae were assigned to *m/z* values as described (Kujawinski and Behn, 2006; Kujawinski et al., 2009). van Krevelen diagrams (Kim et al., 2003) were used to divide the features into compound classes (lignin-like, lipid-like, carbohydrate-like, protein-like, condensed hydrocarbon-like) based on elemental ratios based of oxygen/carbon and hydrogen/carbon as approximated from data within Hedges (1990) and Kim et al. (2003).

3. RESULTS

3.1. Global distribution of Alteromonas AltSIO 16S rRNA

We queried the TARA Oceans Expedition 16S rRNA sequence taxonomy database (Sunagawa et al., 2015), for hits matching *Alteromonas* sp. AltSIO. Sequences with 100% nucleotide identity to AltSIO were found in 128 of 139 total metagenomic samples; matches were found in all 8 major ocean regions sampled, including the North Pacific Ocean, South Pacific Ocean, North Atlantic Ocean, South Atlantic Ocean, Indian Ocean, Southern Ocean, Mediterranean Sea, and the Red Sea in ocean regions spanning from the surface to mesopelagic (Table S1).

3.2. Isolate-specific capacity to metabolize 95 single compounds

A comparative analysis of the AltSIO metabolic profile with four other phylogenetically diverse bacterial strains was conducted by testing the capacity to metabolize 95 individual substrates of broad chemical classes (Fig. 1 and Table S2). Results showed that AltSIO had the capacity to metabolize the broadest range of substrates in all chemical categories relative to other isolates tested (Fig 1). AltSIO oxidized 62 compounds compared with 29, 36, 40, and 18 compounds for TW7, SWAT3, DSS-3, and BBFL-7, respectively. AltSIO showed particular affinity for carbohydrate processing, and oxidized nearly twice as many carbohydrates (mono-, di- and oligosaccharides) as the two other gammaproteobacteria, *Pseudoalteromonas* sp. TW7 and *Vibrio* sp. SWAT3. AltSIO metabolized all L-amino acids tested, but no D-amino acids (Table S2).

3.2. Alteromonas *AltSIO hydrolytic enzyme activity*

AltSIO displayed measureable activity for all enzymes tested except for lipase (Table 1). Yet, the genome contains four distinct lipase genes, one of which is annotated as an outer membrane phospholipase A. Cell-specific enzyme activities were comparable to the range of those measured among 44 marine bacterial strains isolated from the Scripps pier (Martinez et al., 1996) which demonstrates that AltSIO has the capacity to hydrolyze leucine from aminopeptides, and galactose, glucose, and phosphate moieties from organic polymers. While measurements of protease and alkaline phosphatase were relatively low compared with the maximum range observed for other isolates (4.2–3,810, and 0.7– 410 x 10^{-18} mole $cell^{-1}$ hr^{-1}, respectively), AltSIO cell-specific α-D-glucosidase activity was 50% greater than the highest

measured rate among 44 marine bacteria isolated from the same environment (Martinez et al., 1996).

3.3. AltSIO growth response to single sugar amendment in 0.1 µm filtered seawater

After 5 d of incubation, large differences in total cell abundance and viable cell counts were observed between treatments (Table S3). Cell abundance above that measured in the NH_4NO_3-only control was only observed in 4 treatments including sucrose, mannose, galactose, and dilute ZoBell media amendments; within 12 h of substrate addition cell abundance had increased from ~10^5 to ~10^6 mL^{-1}, whereas all other treatments increased only twofold (Fig 2A).

TOC measurements were made to determine the initial ambient TOC concentration of the filtered source water, the total concentration of carbon added in each substrate treatment, and the decrease in TOC concentration after 5 d (Table S3). Because TOC measurements were analyzed from unfiltered samples, decreases in TOC concentration are directly attributed to bacterial carbon respiration. Measurable decreases in TOC after 5 d of incubation were detected in 10 of the 15 treatments (Fig. 2B, Table S3). No measurable changes in TOC concentration were detected in the fucose, rhamnose, sorbose, fructose, or DOC re-feed treatments (Fig. 2B, Table S2). The greatest absolute changes in TOC concentration were observed in sucrose, maltose, galactose, and dilute ZoBell media substrate additions (Fig. 2B, Table S3). Additions of maltose, galactose, and sucrose also resulted in enhanced ("no addition" control subtracted) consumption of TOC below initial pre-amendment concentrations by 6.3, 4.4, and 2.8 µM C, respectively, whereas addition of NH_4NO_3 resulted in enhanced drawdown of only 1.0 µM C (Fig. 2B, Table S3).

3.4. AltSIO incorporation of ^{14}C-labeled sugars and ^{3}H-labeled L-leucine

In two separate experiments, the incorporation of ^{14}C-glucose into cell biomass was below detection when incubated with 100 nM, and 52 nM, respectively for 1 h (Table 2). To confirm that cells were actively synthesizing new biomass, the same batch culture incubated under identical conditions on the same day showed a ^{3}H-L-leucine incorporation rate of 0.4, 0.7, and 3.0 amol cell^{-1} h^{-1} when incubated with 0.7, 2.1, and 6.9 nM leucine, respectively. In a third experiment, longer incubation time (16 h) resulted in detectable, but low incorporation rates (< 0.01 nM C h^{-1}) for both glucose and fructose, but a relatively high incorporation rate of 1.28 ± 0.41 nM C h^{-1} for galactose (Table 2). In a fourth experiment designed to quantify total uptake, incorporation, and respiration (via $^{14}CO_2$ production) of each substrate, total uptake (incorporation + respiration) rates for galactose measured 10-fold higher than those for glucose and fructose (Table 2). Greater than 99% of total glucose and fructose uptake was respired, whereas 68% of galactose uptake was respired, and 32% was incorporated into biomass (Table 2). Also, despite increasing the substrate concentration >10-fold from ~50 nM to ~1 μM between the third and fourth experiment, the proportion of glucose and fructose incorporated remained < 0.01% of the total available pool (Table 2). A 40-fold increase in available galactose concentration resulted in a ~100-fold increase in incorporation rate, but the total proportion of the available substrate pool incorporated into biomass remained ~1% (Table 2).

3.5. Impact of AltSIO on coastal DOM relative to diverse bacterial communities—FT-ICR-MS sample analysis

Three distinct groups were identified based on cluster analysis (Fig 3). These groups can be broadly described as (A) seawater controls without AltSIO incubated for up to 10 d, (B)

seawater samples with AltSIO incubated for up to 10 days, and (C) all treatments incubated for ≥ 40 d. A total of 6,127 features were found shared among all three groups, and between 963-1881 formulas were identified as unique to a single group defined by the cluster analysis (Table 3). However, consideration of compound classes defined by the ratios of carbon, hydrogen, and oxygen revealed small changes (≤ 1.5 % of the total number of features) in the proportion of each compound class across the three groups (Table 3, Fig. S4). Compounds defined as condensed hydrocarbon-like and protein-like were the most prevalent chemical classes within each group, whereas lignin-like, lipid-like, carbohydrate-like compounds represented less < 1% of the total number of unique features in the dataset (Table 3). It is important to note that because group C is comprised of all samples incubated ≥ 40 days, unique features within this cluster cannot be attributed to any single treatment. Treatment-specific comparisons did not show significant differences in the proportion of compounds represented in each compound class, i.e. compositional changes were uniform across treatments.

4. Discussion

4.1. Global distribution of Alteromonas AltSIO 16S rRNA

A search of the TARA Oceans Expedition 16S rRNA taxonomic database, the most comprehensive publicly available environmental metagenomic dataset to date, showed that *Alteromonas* sp. AltSIO, or closely related organisms, are globally distributed (Table S1). In addition, recent studies have shown that closely related taxa that exhibit similar physiological traits are often found in low abundance but are highly active (Campbell et al., 2011; Dupont et al., 2015). The global distribution of AltSIO and significant potential to contribute to

biogeochemical processes (Kim et al., 2015; Pedler et al., 2014) make it an ideal model organism warranting further study.

4.2. AltSIO has the capacity to metabolize a broad suite of substrates

To develop a broad overview of the metabolic repertoire of AltSIO, we tested its ability to utilize 95 different substrates relative to 4 other phylogenetically diverse marine bacteria genera often characterized as generalist species (Lauro et al., 2009). AltSIO stood as the single isolate that oxidized the greatest number of substrates; AltSIO utilized >50% more compounds than *Ruegeria pomeroyi* DSS-3, a model generalist heterotroph within the globally distributed Roseobacter clade (Moran et al., 2004; Newton et al., 2010), and 50% more carbohydrates than the other Alteromonas strain tested (Fig. 1, Table S2). This result supportss the conclusion that AltSIO has the metabolic potential to utilize a wide spectrum of substrates, and provides valuable insight into functional differences between organisms otherwise predicted by genomic inference to occupy the same ecological niche.

4.3. AltSIO selective utilization of neutral sugars for growth in seawater

We then tested the ability of AltSIO to metabolize a suite of neutral sugars because they comprise a substantial fraction of the labile DOM pool (Aluwihare and Repeta, 1999; Goldberg et al., 2011). Incubation with individual sugars in seawater showed that AltSIO preferentially metabolized sucrose, maltose, and galactose for the production of new cells. Surprisingly, cell abundance and changes in TOC concentrations in the glucose-amended treatments were not different from controls, suggesting that AltSIO did not utilize glucose for anabolism or catabolism. In a previous study, it was shown that AltSIO can consume all labile DOM within 5

d of growth in filter-sterilized coastal seawater, but continued incubation (>1 year) did not result in greater measurable DOC drawdown (Pedler et al., 2014). In this study, AltSIO was grown for 5.5 d in ambient DOM to allow for the depletion of labile DOC prior to substrate addition. Enhanced DOC drawdown in treatments amended maltose, galactose, and sucrose (i.e. co-metabolism) beyond levels in NH_4NO_3-amended controls, suggests that the ability of AltSIO to continue to degrade ambient DOC becomes limited by the availability of readily metabolizable preferred carbon sources as opposed to inorganic nutrient availability (Fig 2B, Table S3). This observation is consistent with previously observed DOC drawdown dynamics for bacterioplankton communities in the eastern North Pacific (Cherrier et al., 1996). It is worth noting that studies designed to test co-metabolism of ambient marine DOC and/or the turnover of "labile DOC" often use glucose as the priming substrate (Guenet et al., 2010), but results from this study suggest that glucose may not elicit DOC drawdown by some taxa that otherwise play a central role in marine carbon cycling.

4.4. Uptake, biomass incorporation, and respiration of ^{14}C-radiolabeled glucose, fructose, and galactose

To further understand the apparent selective uptake between glucose, galactose and fructose, we used ^{14}C-radiolabeled substrates to quantify anabolic and catabolic metabolism of each. Results from four separate experiments demonstrate that utilization of glucose or fructose by AltSIO is negligible (Table 2). Conversely, AltSIO showed 10-fold higher total uptake (incorporation + respiration) rates for galactose. The near constant proportional incorporation rate (% h^{-1}) across a 40-fold concentration range suggests the presence of both high and low affinity transporters, thus potentially facilitating rapid consumption of ephemeral organic matter

pulses, and the maintenance of cellular demand through competitive scavenging during low substrate availability. Such adaptations by individual taxa within microbial ecosystems are crucial for understanding the dynamics of ocean carbon flux, and can only be rigorously examined through direct experimental systems such as employed in this study.

Genome analysis showed that AltSIO contains the full suite of genes required for a complete glycolysis metabolic pathway, but an annotated gene was not found for the outer membrane glucose permease protein (KEGG EC 2.7.1.69). However, within the genome an unannotated protein coding sequence was found with ~97% alignment to the consensus glucose phosphotransferase sequence. In *E. coli*, uptake of exogenous glucose by transport across the outer membrane is dependent upon this phosphotransferase enzyme, but galactose can be transported into the cell even in the absence of phosphotransferase activity (Kornberg and Riordan, 1976). AltSIO also displayed a relatively high cell-specific α-glucosidase hydrolysis rate (Table 1) compared to 44 bacterial strains collected from the Scripps pier (Martinez et al., 1996), and genes for both α- and β-glucosidase are present. These enzymes are both involved in the metabolism of galactose, sucrose, starch and other oligosaccharides and polysaccharides through the exohydrolysis of 1-4-α-glucosidic linkages and β-D-glucosyl residues to release α-D-glucose and β-D-glucose, respectively.

The observation that AltSIO does not utilize glucose for anabolic metabolism (and a negligible amount for catabolism), is counterintuitive for several reasons. First, the utilization of glucose requires less cellular energy (adenosine triphosphate, ATP) to metabolize than does galactose. This is because although galactose is an epimer of glucose, once transported into the cell it must be converted to glucose by a series of four enzyme-catalyzed reactions to convert β-D-galactose to UDP-glucose before it can be used in glycolysis (Holden et al., 2003). Second, in

marine and limnetic ecosystems, glucose is widely regarded as a highly labile component of the DOM pool, often measured as the dominant free neutral sugar, and displays rapid turnover rates (Bunte and Simon, 1999; Rich et al., 1997; Rich et al., 1996). Third, AltSIO was shown to rapidly metabolize both maltose (a disaccharide of glucose), and sucrose (a disaccharide comprised of glucose and fructose) for the production of new cell biomass (Fig 2A, 2B). Yet, neither the monomeric form of D-glucose nor D-fructose was utilized (Fig 2A, 2B, Table 2). Lastly, AltSIO is a representative model of generalist (versus as specialists) heterotrophic bacteria based on genome characteristics (Lauro et al., 2009), and observed physiological capacity of Alteromonads to metabolize a wide array of substrates within the DOM pool, as shown here (Fig. 1, Table S2) and elsewhere (Carlson et al., 2004; McCarren et al., 2010; Pedler et al., 2014).

In coastal environments, approximately 10-30% of cells within bacterioplankton communities have been shown to incorporate glucose, with uptake dominated (>50%) by *Alphaproteobacteria*, and *gammaproteobacteria* generally accounting for ~10-20% active glucose incorporating cells (Alonso and Pernthaler, 2006; Alonso-Saez and Gasol, 2007; Elifantz et al., 2005). However, during a seasonal study in the Northwestern Mediterranean, using microautoradiography, Alonso-Saez and Gasol (2007) found that while <10% of gammaproteaobacteria incorporated radiolabel from glucose, 10-60% of those cells incorporated radiolabel from amino acids and ATP, suggesting that while a low proportion of this class of bacteria incorporated glucose the majority were actively incorporating other components of the labile DOM pool. Using stable isotope probing, Nelson and Carlson (2012) found that two genera from the class of gammaproteobacteria, *Alteromonas* and *Marinomonas*, displayed preferential and exclusive incorporation of either glucose or gluconic acid, respectively. A recent

surprising finding showed the inability of several open-ocean SAR11 ecotypes to metabolize all tested monosaccharides yet some coastal ecotypes readily metabolized glucose, only (Schwalbach et al., 2010). Based on these findings it was hypothesized that productive coastal environments, with presumably higher glucose fluxes, select for SAR11 strains with glycolytic capabilities (Schwalbach et al., 2010). This conclusion is in direct contrast to results obtained here for AltSIO which exhibits rapid and efficient consumption of coastal DOC (Pedler et al., 2014), yet strong discrimination against glucose utilization (Table 2). Such specialized utilization of specific substrates from within the labile DOM pool suggests an even finer partitioning of ecological niche space among competing organisms than previously appreciated (Hutchinson, 1957).

We were unable to find a comparable dataset for galactose uptake by marine bacteria (so cannot comment on the generality of our observations) presumably because early work emphasized the apparent dominance of glucose in marine and limnetic systems (Bunte and Simon, 1999; Jorgensen and Jensen, 1994; Rich et al., 1997; Rich et al., 1996). However, recent studies have found that galactose can comprise a substantial fraction of the dissolved combined neutral sugar pool, and is preferentially removed over time relative to glucose, mannose and xylose (Goldberg et al., 2011; Goldberg et al., 2009). These observations along with data presented here suggest that studies focusing on the dynamics of galactose turnover in microbial ecosystems may yield new insights into a reactive component of the marine DOM reservoir.

4.5. Impact of AltSIO on coastal DOM relative to diverse bacterial communities—FT-ICR-MS sample analysis

Ultrahigh resolution mass spectrometry has been used in efforts to link changes in bacterial community composition with observed transformation of DOM chemical composition (Herlemann et al., 2014; Koch et al., 2014; Landa et al., 2014). As a follow up to our recent findings in Pedler et al. (2014), we sought to characterize the effect of *Alteromonas* sp. AltSIO on DOM composition both in isolation and relative to diverse bacterial communities. We note that the percent of condensed hydrocarbon-like compounds detected here is higher than has been observed in other marine systems (Kujawinski et al., 2009; Sleighter and Hatcher, 2008). The identity and source of these compounds remains unknown; however, the proximity of the Scripps pier sampling site to urban influences, such as coastal runoff, and industrial and natural combustion processes could contribute to the observed difference. In time-course incubations with ambient DOM, differences between samples only became detectable in treatments incubated $\geq$ 40 d, yet these differences were not driven by the production or removal of compounds from any single compound class (Table 3, Fig. S4,). This is unsurprising, as we would expect both AltSIO and diverse bacterial consortia to contribute a variety of compounds to these broad compound classes as part of core metabolic processes. Within the context of this study, we interpret these data to suggest: 1) time subjected to bacterial metabolic activity is a stronger determinant of DOM alteration than bacterial community complexity; and 2) the metabolic alteration of ambient DOM by AltSIO in pure culture relative to mixed bacterial assemblages appears uniform across compound classes that are amenable to characterization via FT-ICR-MS. It is important to note that fractionating DOM for compositional analysis and fundamental properties of ESI FT-ICR-MS including ionization bias, our conservative approach focusing on presence/absence peak data instead of relative peak height, coupled with the omnipresent background signal of marine DOM, restrict the depth to which relatively small

changes in chemical composition can be assessed in the context of experimental conditions. Therefore, a quantitative survey of a targeted suite of compounds may be a more fruitful approach to track the flux of microbial metabolites through the marine DOM reservoir.

5. Conclusion

This study characterized the metabolic capacity of a globally distributed model organism, *Alteromonas* sp. AltSIO that has capacity to significantly modulate surface ocean DOC concentrations (Pedler et al., 2014). By focusing on the utilization of carbohydrates, a significant component of the marine DOM reservoir, we have shown that AltSIO displays a strong preference for galactose among all other neutral sugars tested, and two disaccharides, maltose and sucrose. Its lack of D-glucose utilization despite having a complete glycolysis pathway encoded in its genome highlights two important points: 1) functional validation of specific genome-encoded metabolism is critical to understand how individual organisms partition limited resources within the labile DOM pool; and 2) the lack of glucose uptake by an organism previously shown to consume the entire (operationally-defined) labile DOM pool (Pedler et al., 2014) provides an interesting contrast to the vast body of knowledge on glucose uptake as a proxy for labile DOM turnover. Lastly, ultrahigh resolution mass spectrometry showed that both a single bacterial strain grown in isolation and complex bacterial consortia significantly altered ambient DOM from its initial chemical state. Together these findings highlight the important role of conditionally rare taxa (typically rare but occasionally prevalent) (Shade and Gilbert, 2015; Shade et al., 2014), such as AltSIO, to serve as a conduit for the flux and transformation of major fraction of labile marine DOM.

Acknowledgements

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References

Alonso, C. and Pernthaler, J., 2006. Roseobacter and SAR11 dominate microbial glucose uptake in coastal North Sea waters. Environmental Microbiology, 8(11): 2022-2030.

Alonso-Saez, L. and Gasol, J.M., 2007. Seasonal variations in the contributions of different bacterial groups to the uptake of low-molecular-weight compounds in northwestern Mediterranean coastal waters. Applied and Environmental Microbiology, 73(11): 3528-3535.

Aluwihare, L.I. and Repeta, D.J., 1999. A comparison of the chemical characteristics of oceanic DOM and extracellular DOM produced by marine algae. Marine Ecology Progress Series, 186: 105-117.

Aluwihare, L.I., Repeta, D.J. and Chen, R.F., 1997. A major biopolymeric component to dissolved organic carbon in surface sea water. Nature, 387(6629): 166-169.

Amon, R.M.W., Fitznar, H.P. and Benner, R., 2001. Linkages among the bioreactivity, chemical composition, and diagenetic state of marine dissolved organic matter. Limnology and Oceanography, 46(2): 287-297.

Benner, R., 2002. Chemical composition and reactivity. In: D.A. Hansell and C.A. Carlson (Editors), Biogeochemistry of Marine Dissolved Organic Matter. Elsevier Science, USA, pp. 59-90.

Benner, R., Pakulski, J.D., McCarthy, M., Hedges, J.I. and Hatcher, P.G., 1992. Bulk chemical characteristics of dissolved organic matter in the ocean. Science, 255(5051): 1561-4.

Bhatia, M.P., Das, S.B., Longnecker, K., Charette, M.A. and Kujawinski, E.B., 2010. Molecular characterization of dissolved organic matter associated with the Greenland ice sheet. Geochimica Et Cosmochimica Acta, 74(13): 3768-3784.

Bidle, K. and Azam, F., 2001. Bacterial control of silicon regeneration from diatom detritus: Significance of bacterial ectohydrolases and species identity. Limnology and Oceanography, 46(7): 1606-1623.

Bidle, K., Manganelli, M. and Azam, F., 2002. Regulation of oceanic silicon and carbon preservation by temperature control on bacteria. Science, 298(5600): 1980-1984.

Biersmith, A. and Benner, R., 1998. Carbohydrates in phytoplankton and freshly produced dissolved organic matter. Marine Chemistry, 63(1-2): 131-144.

Borch, N.H. and Kirchmann, D.L., 1997. Concentration and composition of dissolved combined neutral sugars (polysaccharides) in seawater determined by HPLC-PAD. Marine Chemistry, 57(1-2): 85-95.

Borsheim, K.Y., Myklestad, S.M. and Sneli, J.A., 1999. Monthly profiles of DOC, mono- and polysaccharides at two locations in the Trondheimsfjord (Norway) during two years. Marine Chemistry, 63(3-4): 255-272.

Bunte, C. and Simon, M., 1999. Bacterioplankton turnover of dissolved free monosaccharides in a mesotrophic lake. Limnology and Oceanography, 44(8): 1862-1870.

Campbell, B., Yu, L., Heidelberg, J. and Kirchman, D., 2011. Activity of abundant and rare bacteria in a coastal ocean. Proceedings of the National Academy of Sciences of the United States of America, 108(31): 12776-12781.

Carlson, C. and Ducklow, H., 1996. Growth of bacterioplankton and consumption of dissolved organic carbon in the Sargasso Sea. Aquatic Microbial Ecology, 10(1): 69-85.

Carlson, C., Ducklow, H. and Michaels, A., 1994. Annual flux of dissolved organic carbon from the euphotic zone in the northwestern sargasso sea. Nature, 371(6496): 405-408.

Carlson, C. et al., 2004. Interactions among dissolved organic carbon, microbial processes, and community structure in the mesopelagic zone of the northwestern Sargasso Sea. Limnology and Oceanography, 49(4): 1073-1083.

Carlson, C.A., 2002. Production and removal processes. In: D.A. Hansell and C.A. Carlson (Editors), Biogeochemistry of marine dissolved organic matter. Elsevier Science, USA, pp. 91-151.

Carlson, C.A. and Hansell, D.A., 2015. Chapter 3 - DOM Sources, Sinks, Reactivity, and Budgets. In: D.A.H.A. Carlson (Editor), Biogeochemistry of Marine Dissolved Organic Matter (Second Edition). Academic Press, Boston, pp. 65-126.

Cherrier, J., Bauer, J. and Druffel, E., 1996. Utilization and turnover of labile dissolved organic matter by bacterial heterotrophs in eastern north Pacific surface waters. Marine Ecology Progress Series, 139(1-3): 267-279.

Cowie, G.L. and Hedges, J.I., 1994. Biochemical indicators of diagenetic alteration in natural organic matter mixtures. Nature, 369(6478): 304-307.

Dittmar, T., Koch, B., Hertkorn, N. and Kattner, G., 2008. A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. Limnology and Oceanography-Methods, 6: 230-235.

Druffel, E.R.M., Williams, P.M., Bauer, J.E. and Ertel, J.R., 1992. Cycling of Dissolved and Particulate Organic-Matter in the Open Ocean. Journal of Geophysical Research-Oceans, 97(C10): 15639-15659.

Ducklow, H.W., 1999. The bacterial component of the oceanic euphotic zone. Fems Microbiology Ecology, 30(1): 1-10.

Dupont, C.L. et al., 2015. Genomes and gene expression across light and productivity gradients in eastern subtropical Pacific microbial communities. ISME J, 9(5): 1076-92.

Elifantz, H., Malmstrom, R.R., Cottrell, M.T. and Kirchman, D.L., 2005. Assimilation of polysaccharides and glucose by major bacterial groups in the Delaware Estuary. Applied and Environmental Microbiology, 71(12): 7799-7805.

Fuhrman, J. and Azam, F., 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: Evaluation and field results. Marine Biology, 66(2): 109-120.

Goldberg, S.J., Carlson, C.A., Brzezinski, M., Nelson, N.B. and Siegel, D.A., 2011. Systematic removal of neutral sugars within dissolved organic matter across ocean basins. Geophysical Research Letters, 38.

Goldberg, S.J., Carlson, C.A., Hansell, D.A., Nelson, N.B. and Siegel, D.A., 2009. Temporal dynamics of dissolved combined neutral sugars and the quality of dissolved organic matter in the Northwestern Sargasso Sea. Deep-Sea Research Part I-Oceanographic Research Papers, 56(5): 672-685.

Guenet, B., Danger, M., Abbadie, L. and Lacroix, G., 2010. Priming effect: bridging the gap between terrestrial and aquatic ecology. Ecology, 91(10): 2850-2861.

Hansell, D.A., 2005. Dissolved organic carbon reference material program. EOS, Transactions American Geophysical Union, 86(35): 318-318.

Hansell, D.A., 2013. Recalcitrant dissolved organic carbon fractions. Annual review of marine science, 5: 421-45.

Hedges, J.I., 1990. Compositional Indicators of Organic-Acid Sources and Reactions in Natural Environments. Organic Acids in Aquatic Ecosystems, 48: 43-63.

Herlemann, D.P.R. et al., 2014. Uncoupling of Bacterial and Terrigenous Dissolved Organic Matter Dynamics in Decomposition Experiments. Plos One, 9(4).

Holden, H.M., Rayment, I. and Thoden, J.B., 2003. Structure and function of enzymes of the Leloir pathway for galactose metabolism. Journal of Biological Chemistry, 278(45): 43885-43888.

Hoppe, H.G., 1983. Significance of exoenzymatic activities in the ecology of brackish water - measurements by means of methylumbelliferyl substrates. Marine Ecology Progress Series, 11(3): 299-308.

Hoppe, H.G., Kim, S.J. and Gocke, K., 1988. Microbial decomposition in aquatic environments combined process of extracellular enzyme activity and substrate uptake. Applied and Environmental Microbiology, 54(3): 784-790.

Hugoni, M. et al., 2013. Structure of the rare archaeal biosphere and seasonal dynamics of active ecotypes in surface coastal waters. Proceedings of the National Academy of Sciences of the United States of America, 110(15): 6004-6009.

Hutchinson, G.E., 1957. Concluding remarks, Cold Spring Harbor Symp Quant Biol, pp. 414-427.

Ittekkot, V., Brockmann, U., Michaelis, W. and Degens, E.T., 1981. Dissolved Free and Combined Carbohydrates during a Phytoplankton Bloom in the Northern North Sea. Marine Ecology Progress Series, 4(3): 299-305.

Jorgensen, N.O.G. and Jensen, R.E., 1994. Microbial Fluxes of Free Monosaccharides and Total Carbohydrates in Fresh-Water Determined by Pad-Hplc. Fems Microbiology Ecology, 14(1): 79-93.

Karl, D.M., Hebel, D.V., Bjorkman, K. and Letelier, R.M., 1998. The role of dissolved organic matter release in the productivity of the oligotrophic North Pacific Ocean. Limnology and Oceanography, 43(6): 1270-1286.

Kido Soule, M.C., Longnecker, K., Giovannoni, S.J. and Kujawinski, E.B., 2010. Impact of instrument and experiment parameters on reproducibility of ultrahigh resolution ESI FT-ICR mass spectra of natural organic matter. Organic Geochemistry, 41(8): 725-733.

Kielbasa, S.M., Wan, R., Sato, K., Horton, P. and Frith, M.C., 2011. Adaptive seeds tame genomic sequence comparison. Genome Res, 21(3): 487-93.

Kim, M.J. et al., 2015. Bacteria-driven production of alkyl nitrates in seawater. Geophysical Research Letters, 42(2): 597-604.

Kim, S., Kramer, R.W. and Hatcher, P.G., 2003. Graphical method for analysis of ultrahigh-resolution broadband mass spectra of natural organic matter, the van Krevelen diagram. Analytical Chemistry, 75(20): 5336-5344.

Kirchman, D., Suzuki, Y., Garside, C. and Ducklow, H., 1991. High turnover rates of dissolved organic carbon during a spring phytoplankton bloom. Nature, 352(6336): 612-614.

Kirchman, D.L. et al., 2001. Glucose fluxes and concentrations of dissolved combined neutral sugars (polysaccharides) in the Ross Sea and Polar Front Zone, Antarctica. Deep-Sea Research Part Ii-Topical Studies in Oceanography, 48(19-20): 4179-4197.

Koch, B.P., Kattner, G., Witt, M. and Passow, U., 2014. Molecular insights into the microbial formation of marine dissolved organic matter: recalcitrant or labile? Biogeosciences, 11(15): 4173-4190.

Koch, B.P., Witt, M.R., Engbrodt, R., Dittmar, T. and Kattner, G., 2005. Molecular formulae of marine and terrigenous dissolved organic matter detected by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. Geochimica Et Cosmochimica Acta, 69(13): 3299-3308.

Kornberg, H.L. and Riordan, C., 1976. Uptake of galactose into *Escherichia coli* by facilitated diffusion. Journal of General Microbiology, 94(MAY): 75-89.

Kujawinski, E.B. and Behn, M.D., 2006. Automated analysis of electrospray ionization Fourier transform ion cyclotron resonance mass spectra of natural organic matter. Analytical Chemistry, 78(13): 4363-4373.

Kujawinski, E.B. et al., 2009. Identification of possible source markers in marine dissolved organic matter using ultrahigh resolution mass spectrometry. Geochimica Et Cosmochimica Acta, 73(15): 4384-4399.

Landa, M. et al., 2014. Phylogenetic and structural response of heterotrophic bacteria to dissolved organic matter of different chemical composition in a continuous culture study. Environmental Microbiology, 16(6): 1668-1681.

Lauro, F. et al., 2009. The genomic basis of trophic strategy in marine bacteria. Proceedings of the National Academy of Sciences of the United States of America, 106(37): 15527-15533.

Letscher, R.T., Hansell, D.A., Carlson, C.A., Lumpkin, R. and Knapp, A.N., 2013. Dissolved organic nitrogen in the global surface ocean: Distribution and fate. Global Biogeochemical Cycles, 27: 1-13.

Long, R. and Azam, F., 2001. Antagonistic interactions among marine pelagic bacteria. Applied and Environmental Microbiology, 67(11): 4975-4983.
Markowitz, V.M. et al., 2009. IMG ER: a system for microbial genome annotation expert review and curation. Bioinformatics, 25(17): 2271-2278.
Martinez, J., Smith, D.C., Steward, G.F. and Azam, F., 1996. Variability in ectohydrolytic enzyme activities of pelagic marine bacteria and its significance for substrate processing in the sea. Aquatic Microbial Ecology, 10(3): 223-230.
McCarren, J. et al., 2010. Microbial community transcriptomes reveal microbes and metabolic pathways associated with dissolved organic matter turnover in the sea. Proceedings of the National Academy of Sciences of the United States of America, 107(38): 16420-16427.
McCarthy, M., Hedges, J. and Benner, R., 1996. Major biochemical composition of dissolved high molecular weight organic matter in seawater. Marine Chemistry, 55(3-4): 281-297.
Moran, M.A. et al., 2004. Genome sequence of Silicibacter pomeroyi reveals adaptations to the marine environment. Nature, 432(7019): 910-3.
Nelson, C. and Carlson, C., 2012. Tracking differential incorporation of dissolved organic carbon types among diverse lineages of Sargasso Sea bacterioplankton. Environmental Microbiology, 14(6): 1500-1516.
Newton, R.J. et al., 2010. Genome characteristics of a generalist marine bacterial lineage. Isme Journal, 4(6): 784-798.
Pakulski, J.D. and Benner, R., 1994. Abundance and distribution of carbohydrates in the ocean. Limnology and Oceanography, 39(4): 930-940.
Pedler, B.E., Aluwihare, L.I. and Azam, F., 2014. Single bacterial strain capable of significant contribution to carbon cycling in the surface ocean. Proceedings of the National Academy of Sciences of the United States of America, 111(20): 7202-7207.
Porter, K. and Feig, Y., 1980. The use of DAPI for identifying and counting aquatic microflora. Limnology and Oceanography, 25(5): 943-948.
Repeta, D.J. and Aluwihare, L.I., 2006. Radiocarbon analysis of neutral sugars in high-molecular-weight dissolved organic carbon: Implications for organic carbon cycling. Limnology and Oceanography, 51(2): 1045-1053.
Rich, J., Gosselin, M., Sherr, E., Sherr, B. and Kirchman, D.L., 1997. High bacterial production, uptake and concentrations of dissolved organic matter in the Central Arctic Ocean. Deep-Sea Research Part Ii-Topical Studies in Oceanography, 44(8): 1645-1663.
Rich, J.H., Ducklow, H.W. and Kirchman, D.L., 1996. Concentrations and uptake of neutral monosaccharides along 140 degrees W in the equatorial Pacific: Contribution of glucose to heterotrophic bacterial activity and the DOM flux. Limnology and Oceanography, 41(4): 595-604.
Schwalbach, M.S., Tripp, H.J., Steindler, L., Smith, D.P. and Giovannoni, S.J., 2010. The presence of the glycolysis operon in SAR11 genomes is positively correlated with ocean productivity. Environmental Microbiology, 12(2): 490-500.
Shade, A. and Gilbert, J.A., 2015. Temporal patterns of rarity provide a more complete view of microbial diversity. Trends Microbiol, 23(6): 335-340.
Shade, A. et al., 2014. Conditionally rare taxa disproportionately contribute to temporal changes in microbial diversity. MBio, 5(4): e01371-14.
Sleighter, R.L. and Hatcher, P.G., 2008. Molecular characterization of dissolved organic matter (DOM) along a river to ocean transect of the lower Chesapeake Bay by ultrahigh

resolution electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. Marine Chemistry, 110(3-4): 140-152.
Sunagawa, S. et al., 2015. Ocean plankton. Structure and function of the global ocean microbiome. Science, 348(6237): 1261359.
Tada, Y. et al., 2011. Differing Growth Responses of Major Phylogenetic Groups of Marine Bacteria to Natural Phytoplankton Blooms in the Western North Pacific Ocean. Applied and Environmental Microbiology, 77(12): 4055-4065.
Tada, Y., Taniguchi, A., Sato-Takabe, Y. and Hamasaki, K., 2012. Growth and succession patterns of major phylogenetic groups of marine bacteria during a mesocosm diatom bloom. Journal of Oceanography, 68(4): 509-519.

Figure legends:

Figure 1. Summarized results for oxidation of 95 individual substrates by AltSIO relative to phylogenetically diverse bacterial strains. Full compound-specific response of each isolate is listed in Table S2.

Figure 2. AltSIO growth response to neutral sugar amended 0.1 μm-filtered seawater. A) Bacterial abundance measured by direct cell counts. B) Sugar treatment-specific metabolic carbon partitioning measured by changes in bacterial biomass and TOC concentration. Dashed line indicates DOC consumed above measured carbon addition.

Figure 3. Cluster dendrogram of 22 samples analyzed by FT-ICR-MS, distance calculated by Bray-Curtis measure and Ward's linkage method. Abbreviations: C, control; S, seawater community; A, AltSIO; *, sampled from 2012 DOC drawdown experiment as described (Pedler et al., 2014). Numbers indicate days incubated. Lowercase letters indicate replicates.

Figure 1

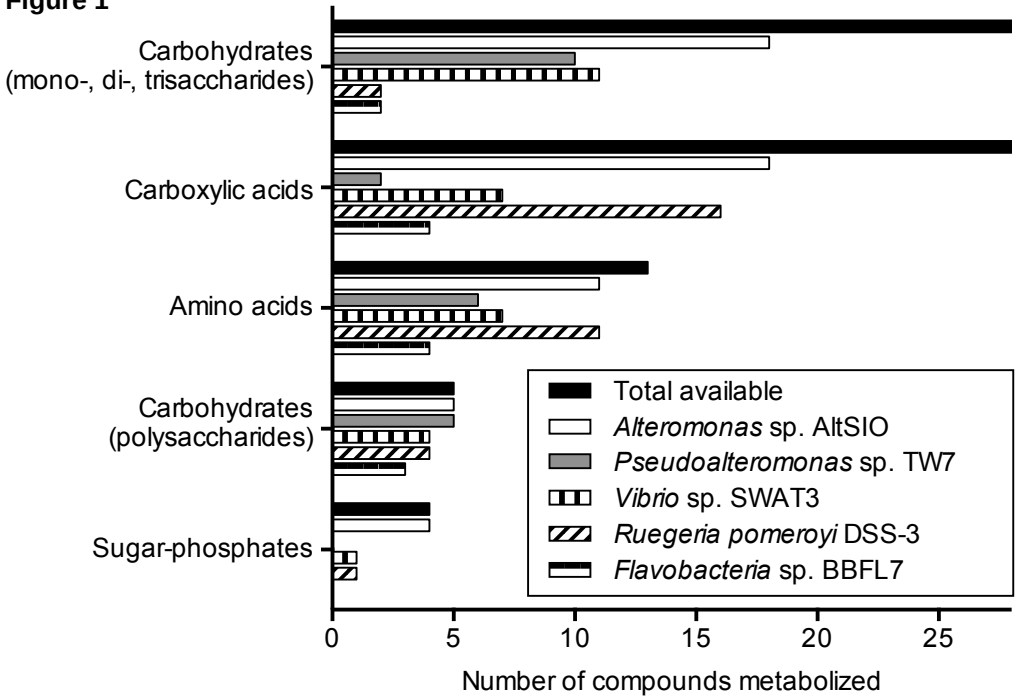
Carbohydrates (mono-, di-, trisaccharides)
Carboxylic acids
Amino acids
Carbohydrates (polysaccharides)
Sugar-phosphates
Total available
Alteromonas sp. AltSIO
Pseudoalteromonas sp. TW7
Vibrio sp. SWAT3
Ruegeria pomeroyi DSS-3
Flavobacteria sp. BBFL7
0
5
10
15
20
25
Number of compounds metabolized

Figure 2

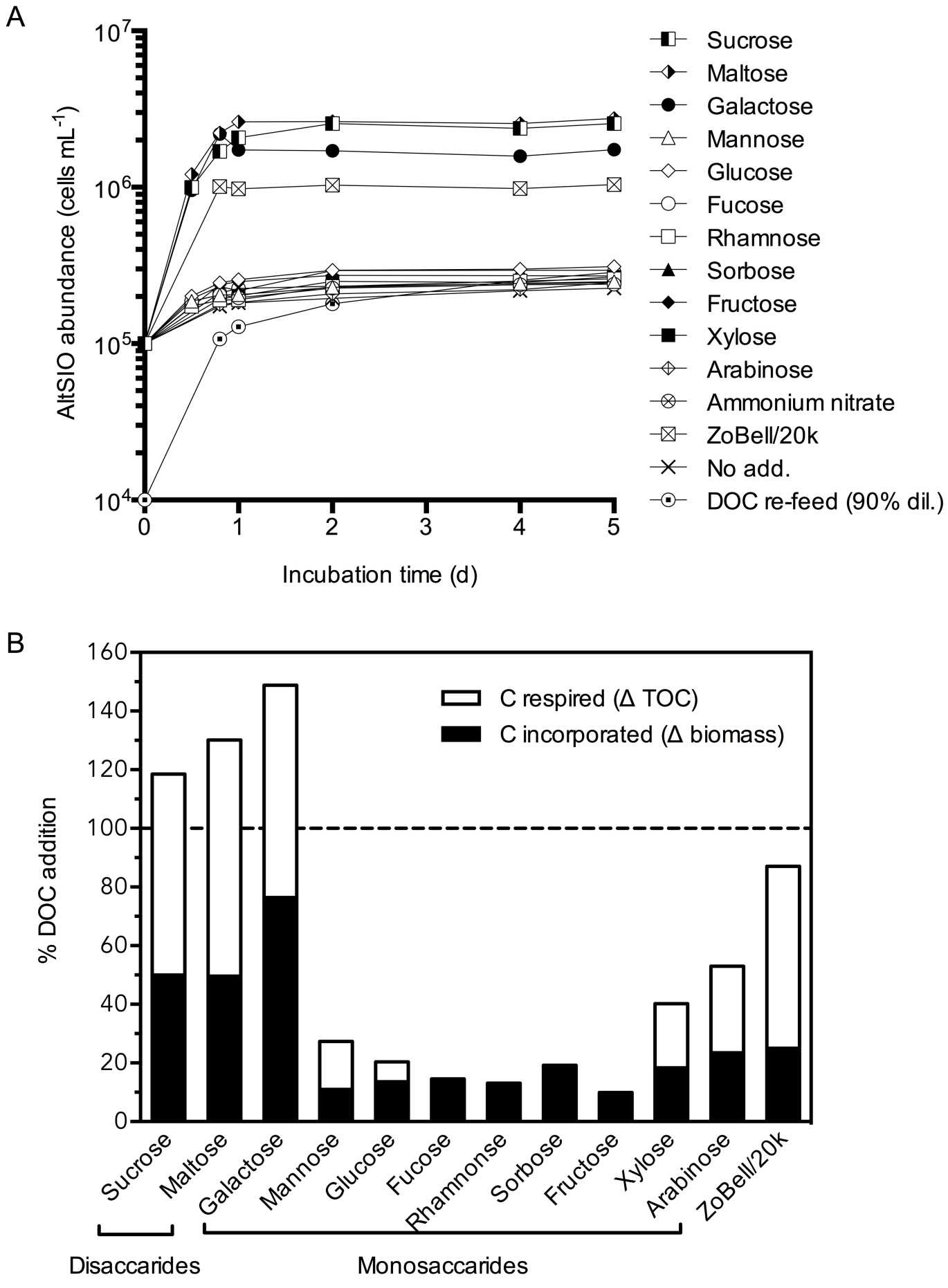
A
AltSIO abundance (cells mL-1)
10^7
10^6
10^5
10^4
0
1
2
3
4
5
Incubation time (d)
Sucrose
Maltose
Galactose
Mannose
Glucose
Fucose
Rhamnose
Sorbose
Fructose
Xylose
Arabinose
Ammonium nitrate
ZoBell/20k
No add.
DOC re-feed (90% dil.)
B
% DOC addition
160
140
120
100
80
60
40
20
0
C respired (Δ TOC)
C incorporated (Δ biomass)
Sucrose
Maltose
Galactose
Mannose
Glucose
Fucose
Rhamnonse
Sorbose
Fructose
Xylose
Arabinose
ZoBell/20k
Disaccarides
Monosaccarides

Figure 3

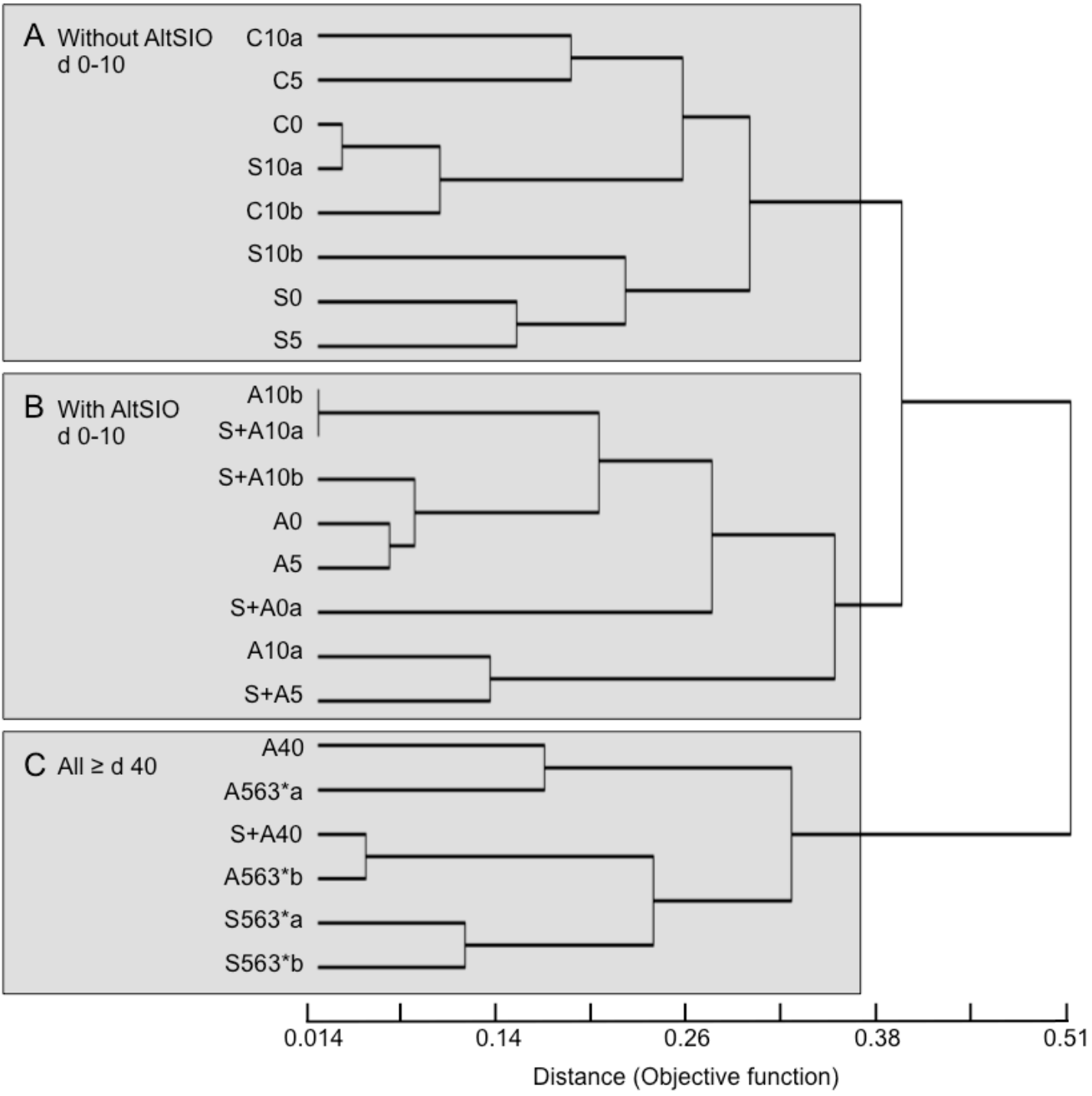
A Without AltSIO
d 0-10
C10a
C5
C0
S10a
C10b
S10b
S0
S5
B With AltSIO
d 0-10
A10b
S+A10a
S+A10b
A0
A5
S+A0a
A10a
S+A5
C All ≥ d 40
A40
A563*a
S+A40
A563*b
S563*a
S563*b
0.014
0.14
0.26
0.38
0.51
Distance (Objective function)

Table 1. AltSIO cell-specific hydrolytic enzyme activity

Enzyme	Hydrolysis rate (10^{-18} mole cell^{-1} hr^{-1})	
	mean	s.d.
Leucine aminopeptidase	46.3	1.9
Alkaline phosphatase	35.8	0.8
β-D-galactosidase	18.8	3.8
α-D-glucosidase	12.5	4.3
lipase	n.d.	

Table 2. Incorporation and respiration rates of ^{14}C-radiolabeled sugars by *Alteromonas* sp. AltSIO.

Substrate	Addition (μM C)	C flux rate (nM · h^{-1})		Proportion utilized (% · h^{-1})	
		Incorporated	Respired	Incorporated	Respired
Glucose	0.60	b.d.		b.d.	
Glucose	0.60	b.d.		b.d.	
Glucose	0.31	0.006 ± 0.002		< 0.01	
Glucose	6.96	0.28 ± 0.01	28.65 ± 0.94	< 0.01	0.4 ± 0.0
Fructose	0.26	0.001 ± 0.002		< 0.01	
Fructose	5.65	0.11 ± 0.01	30.31 ± 1.61	< 0.01	0.5 ± 0.0
Galactose	0.14	1.28 ± 0.41		0.9 ± 0.3	
Galactose	7.72	95.25 ± 4.56	205.54 ± 3.88	1.2 ± 0.1	2.7 ± 0.1

Values with paired incorporation and respiration data are mean ± standard deviation of 3 replicate bottle incubations. All other values with associated error are mean ± standard deviation of 3 to 4 methodological vial replicates. Abbreviations; b.d., below detection.

Table 3. Total unique formula assignments and their proportional classification into select compound classes as defined by van Krevelen diagrams (Fig 4). Sample clusters defined in Fig. 3.

Sample cluster	Avg. number unique compounds	% Compounds in each chemical class, mean (min—max)				
		Condensed hydrocarbon	Protein	Lignin	Lipid	Carbohydrates
A. Without AltSIO d 0-10	6068.3	14.5 (14.1 — 15.3)	4.7 (4.5 — 4.9)	0.9 (0.8 — 1.0)	0.6 (0.6 — 0.7)	0.8 (0.7 — 0.9)
B. With AltSIO d 0-10	5528.9	15.3 (14.7 — 16.1)	4.6 (4.3 — 4.8)	1.0 (0.9 — 1.1)	0.6 (0.6 — 0.7)	0.7 (0.6 — 0.9)
C. All ≥ d 40	5028.3	14.8 (14.4 — 15.4)	4.8 (4.4 — 5.1)	1.0 (0.9 — 1.0)	0.7 (0.6 — 0.9)	0.7 (0.6 — 0.9)