Title: **Biological Cycling of Volatile Organic Carbon by Phytoplankton and Bacterioplankton**

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Running Title: **VOC cycling by marine plankton**

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ABSTRACT

Acetaldehyde, methanol, acetone, and isoprene are important reactive volatile organic compounds (VOCs) in the oceans that partition to the atmosphere in significant amounts. Reports of potentially high rates of VOC turnover in the North Atlantic suggested that both biotic and abiotic processes are involved. The biological basis for VOC cycling by ocean plankton is unknown, but is potentially important because of VOC contributions to carbon cycle budgets and atmospheric chemistry. We designed dynamic stripping chambers that coupled to a proton transfer reaction mass spectrometer to measure VOC production and consumption by cultured phytoplankton and bacterioplankton. The diatom, *Thalassiosira pseudonana*, produced acetaldehyde in a light-dependent manner. Acetaldehyde was oxidized by the chemoheterotrophic bacterium, *Pelagibacter*, at rates that suggest that most acetaldehyde is recycled in the ocean before escaping to the atmosphere. These results show that field observations of acetaldehyde turnover reported previously could be explained by biological activity. Rates of production by phytoplankton cultures of methanol, acetone, and isoprene were also measured. These findings support the conclusion that VOCs are a conduit for carbon transfer directly from phytoplankton to bacterioplankton, with the remainder available for escape to the atmosphere.

Introduction.

Volatile organic compounds (VOCs) are a diverse group of chemicals that are produced by biotic and abiotic mechanisms on the planet surface. VOCs have high vapor pressures that facilitate their entry into the atmosphere where they contribute to formation of hydroxyl radicals (Jacob et al. 2002; Singh et al. 1995). Attention has focused on atmospheric VOCs (including acetaldehyde, acetone, methanol, alkenes and alkanes, methyl halides, DMS, and isoprene) because they are implicated in the formation of atmospheric peroxy acetyl nitrate and ozone (Folkins and Chatfield 2000) and are climate-active gases. The oceans are recognized as a major source of atmospheric VOCs (Fischer et al. 2012), but the chemical and biochemical mechanisms producing and consuming oceanic VOCs are not well understood. Aside from their potential significance to atmospheric chemistry, VOCs are important to oceanographers because these compounds make uncertain, but potentially significant contributions to carbon budgets. Only recently has information about the cycling of VOCs in the ocean surface layer become available (Dixon et al. 2011a; Dixon et al. 2011b; Halsey et al. 2012; Sun et al. 2011). The data suggest that a significant fraction of biological carbon oxidation activity in the ocean surface layer is fueled by VOC turnover, but the number of measurements made so far is relatively small.

Acetaldehyde, the primary focus of this report, is produced in ocean surface samples at a rate of 25–98 nmol L^{-1} d^{-1} (Dixon et al. 2013) with surface water concentrations ranging from 4-37 nM (Beale et al. 2015). Dixon et al. (2013) concluded that abiotic photochemistry could account for as much as 68% of ocean surface acetaldehyde production, leaving unknown biological processes, or precipitation, as the likely sources of the remaining acetaldehyde production. Acetaldehyde is most well known to biochemists as a product of ethanol oxidation in

animals, or a fermentation product of organic matter catabolism in anaerobic ecosystems. In marine systems, acetaldehyde is produced by photooxidation of Chromophoric Dissolved Organic Matter (CDOM) from terrestrially-derived plant matter or degrading algal biomass (de Bruyn et al. 2011; Kieber et al. 1989). Algae have been observed to release acetaldehyde that accumulates in headspace gasses (Collins and Bean 1963; Collins and Kalnins 1965; Collins and Kalnins 1966), but neither the biochemical mechanisms, phylogenetic distribution, nor biological function of acetaldehyde production are known for ocean surface microbiota.

One of the reasons that information about the microbial ecology of low molecular weight VOC's, such as acetaldehyde, methanol, and acetone, has not accumulated rapidly is they are challenging molecules to accurately measure, especially at low concentrations. New information about these molecules is arising from the application of proton-transfer reaction mass spectrometers (PTR-MS), which detect VOCs with high proton affinities by ionizing them with hydronium ions.

The total contribution of VOCs to the ocean carbon cycle has not been constrained accurately for the purpose of building ocean carbon budgets. For example, it has been suggested that systematic underestimates of VOC delivery to heterotrophic bacterioplankton could explain why some ocean regions appear to respire more organic carbon than they are estimated to produce (Duarte et al. 2013); for example, overlooked VOC production by phytoplankton could result in underestimates of primary production (Halsey and Jones 2015). Whether these productive and consumptive processes are important globally depends upon their magnitude.

The goal of this study was to explore the biology underlying VOC production and consumption by microbes in the ocean surface. For this purpose, we adopted technical approaches widely used by atmospheric chemists, but modified them for biological experiments

with cultured cells under controlled conditions. We report light-dependent production of acetaldehyde by a diatom at rates that are similar to previous reports of DMS production. We also report acetaldehyde consumption by bacterioplankton of the SAR11 clade at rates that far exceed rates of methanol and formaldehyde oxidation by SAR11, but that are similar to rates of pyruvate oxidation by surface water communities dominated by SAR11 (Sun et al. 2011). We further identify genes that underlie acetaldehyde metabolism in SAR11 that are very abundant in the global oceans. Light-dependent production rates for methanol and acetone are also reported for a diatom and a green alga. This work confirms that cellular activities support a planktonic VOC cycle. In particular, acetaldehyde and methanol are model examples of microbial loop functions that have been generally hidden from studies on biogeochemical cycles because the established methods for measuring dissolved organic carbon (DOC) effectively remove VOCs from DOC samples. This work highlights acetaldehyde as an example VOC that is cycled by important members of the marine microbial community. These laboratory-based results are important for improving estimates of VOC emissions from the oceans (Gantt et al. 2009; Guenther et al. 1995; Williams et al. 2004).

Methods

Culture conditions: Dunaliella tertiolecta (CCMP 1320) and *Thalassiosira pseudonana* (CCMP 1335) were grown at 20°C in 1 l batch cultures in f/2 medium (Guillard 1975). Axenic cultures were kept in exponential phase by removal of culture and addition of fresh medium. Growth irradiance was kept constant at ~60 µmol photons m^{-2} s^{-1}. Chlorophyll concentrations were determined from 4 ml (*D. tertiolecta*) and 8 ml (*T. pseudonana*) samples filtered onto 25 mm glass fiber filters (Whatman GF/F) in triplicate. Filters were extracted for 24 h in 2.5 ml 90%

acetone and the extract measured spectrophotometrically for chlorophyll *a* (Chl*a*) concentration using the equations of Mitchell et al. (2003). Chl*a* concentrations of cultures transferred directly into the dynamic stripping chambers for VOC production measurements were 0.14 – 0.19 μg ml^{-1} for *D. tertiolecta* and 0.03 – 0.04 μg ml^{-1} for *T. pseudonana.* Cultures were neither concentrated nor diluted prior to VOC measurement.

SAR11 cultures were grown in seawater-based medium with excess vitamins (Steindler et al. 2011) and amended with 1 mM NH_4Cl, 100 μM KH_2PO_4, 1 μM $FeCl_3$, 100 μM pyruvate, 50 μM glycine, and 50 μM methionine. Cells were counted by staining with the DNA stain SYBR Green I (Invitrogen) for one hour, followed by enumeration with a flow cytometer (Guava Technologies) (Tripp et al. 2008). Cells were harvested in log phase by high speed centrifugation (1 hour at 43,700 g, 10º C), resuspended to 5 x 10^7 cells ml^{-1} in fresh growth medium, and transferred to the dynamic stripping chambers for VOC measurement.

PTR-MS measurement of plankton VOC production and consumption: Cultures (100 ml) or media only controls were added to dynamic stripping chambers as described above. CO_2 (380 ppm) in synthetic air was continuously supplied to the chambers through sintered glass frits (2-2.5 μm) at the base of 200 ml polycarbonate tubes (Fig 1). Dynamic stripping chambers were kept at 20°C and light was supplied by an LED system at 0, 50, 100, 150 or 1500 μmol photons m^{-2} s^{-1} as measured by a quantum meter (model QSL-100; Biospherical Instruments, San Diego, CA, USA) fitted with a 4π spherical quantum sensor.

Acetaldehyde, methanol, acetone, and isoprene were measured using proton transfer reaction – mass spectrometry (PTR-MS, Ionicon, Austria). The gas in the headspace of only one dynamic stripping chamber can be measured by the PTR-MS at a time. Therefore, during

incubations, the gas in the headspace of each chamber was directed to the PTR-MS for 1-3 min before a multiport valve automatically switched to sample the next chamber in the sequence. Headspace gas enters the PTR-MS where compounds that are constituents of the gas are protonated by hydronium ions (H_3O^+) if the affinity of the compound for protons is greater than that of water. Acetaldehyde, methanol, acetone, and isoprene were detected by the quadrupole mass spectrometer at their molecular masses +1; 45, 33, 59, and 69, respectively. Mixing ratios were calculated from the observed ion signals using widely accepted methods described elsewhere (de Gouw and Warneke 2007). A standard mixture of acetaldehyde, methanol, acetone, and isoprene was used to calibrate the measurements. Calibrations using standard mixtures results in PTR-MS measurement accuracy of 20%. This accuracy compares favorably to other methods (i.e., GC applications) that do not allow for the fast-response measurements of VOCs obtained by PTR-MS (de Gouw and Warneke 2007).

D. tertiolecta was exposed to two light intensities for ~40 min each and no light for ~60 min. *T. pseudonana* was exposed to three light intensities of increasing intensity for 15 min each and one high light intensity for ~30 min. These light exposure durations allowed for exponential functions to be applied to the output signals to determine the VOC production rate during culture exposure to each light intensity. Light-dependent VOC production was calculated as the difference between VOC concentration measured in cultures exposed to light and that measured in cultures in the dark. Light-dependent VOC production in phytoplankton suggests that VOCs are produced as a result of active photosynthetic metabolism as opposed to respiratory activity in the dark.

^{14}C-acetaldehyde uptake and oxidation assays: *Pelagibacter* cells were harvested in log phase by centrifugation (1 hour at 43,700 g, 10°C) and resuspended in fresh medium to about 5 x 10^7 cells ml^{-1}. 100 nM ^{14}C-acetaldehyde (10 mCi/mmol) were added to both ‘‘Live’’ and ‘‘Killed’’ culture samples; negative controls (‘‘killed’’) were incubated in 10% formalin for 1 hour before the addition of the isotope to the sample. Inoculated cultures (4 mL) were aliquoted into 40 mL glass vials that were then sealed with teflon lined stoppers. Samples were incubated in the dark at 17 °C. At each time point, reagents were added to cultures using syringes inserted through stoppers.

For ^{14}C incorporation, 100% w/v cold TCA was added to final concentration of 10%. To precipitate $^{14}CO_2$, 0.2 mL 1 M NaOH, 0.5 mL 0.1 M Na_2CO_3 and 1 mL 1 M $BaCl_2$ were added to form $Ba^{14}CO_3$ and BaOH (Halsey et al. 2012). All samples were collected by filtration after incubation at 4°C for 12 hours. Precipitates were collected on 0.2 μm nitrocellulose GSWP filters (Millipore, Billerica, MA), washed three times with 3 ml of ice-cold 5% w/v TCA (^{14}C incorporation) or 3 ml of ice-cold fresh SAR11 growth medium (^{14}C oxidation) and transferred to scintillation vials. Filters were transferred to vials containing 5 mL Ultima GoldTM XR scintillation fluid (Perkin-Elmer), shaken vigorously, and kept in dark overnight before counting (Beckman LS-6500 liquid scintillation counter).

Identification of potential SAR11 Acetaldehyde dehydrogenases (ALDH) genes: Nineteen SAR11 genomes were downloaded from Integrated Microbial Genomes database (IMG: http://img.jgi.doe.gov/) in FASTA format. A profile Hidden Markov Model (HMM) was built for previously identified bacterial ALDH sequences (including *E. coli aldA*, *aldB*, *aldH*, *ydcW*, SSADH etc.) by using hmmbuild (http://hmmer.org/, v3.1b2), and the resulting protein profile HMM was searched against amino acid sequences of 19 SAR11 genomes using hmmsearch. 119

putative ALDH genes were identified. ALDH genes sharing more than 60% amino acid sequence identity were placed within a subfamily, and those with sequences less than 40% identity represent a new family (Sophos and Vasiliou 2003; Vasiliou et al. 1999).

Reciprocal best-blast recruitment of GOS translated peptide sequences: Translated amino acid sequences of 17 potential ALDHs as well as the recombinase A (RecA) protein from SAR11 strains, HTCC7211 and HTCC1062 were initially compared to the Global Ocean Survey (Rusch et al. 2007) translated peptide database using blastp (version 2.2.26+; options: -evalue 1 –outfmt 5 -max_target_seqs 1000000000). All returned peptide sequences were then compared using blastp against a version of the refseq 65 database with all known Single amplified genomes (SAGs) removed (options: -outfmt 5 –max_target_seqs 1). Reciprocal best-blast hits were recorded if the accession for the top hit for a given read matched a member of the original set of query sequences. A series of python scripts was used to organize the queuing blast jobs, as well as the organization and formatting of results. A single blastp job was used for the initial search, and the reciprocal blast search was divided into 2890 individual blast jobs of 24 sequences each, which were divided across the machines available at Center for Genome Research and Biocomputing (CGRB) at Oregon State University.

Global scale acetaldehyde cycling. Global chlorophyll was based on R2014 Moderate-Resolution Imaging Spectroradiometer (MODIS) remote sensing 8-day files, from mid-2002 through mid-2015 (totaling 601 files) and yielded an average value of 4.33×10^{12} g Chl with a standard deviation of 0.27×10^{12} g Chl. Global chlorophyll was estimated by integrating the surface values down to the euphotic depth. Data were obtained from the Ocean Productivity web

site at Oregon State University (www.science.oregonstate.edu/ocean.productivity/). Diatoms are 27% of global chlorophyll (Bopp et al. 2005). Total acetaldehyde produced ($7.7 \times 10^{11} \pm 1.6 \times 10^{11}$ mol d^{-1}) was calculated using the rate of production at 150 µmol photons m^{-2} s^{-1} (a light intensity that is sub-saturating for the mixed layer) for 6 h per day, while SAR11 consumes acetaldehyde 24 h per day (totaling $7.8 \times 10^{13} \pm 1.9 \times 10^{13}$ mol d^{-1}). Uncertainty was calculated using propagation of error with VOC production and consumption rates having 20% accuracy and the global estimate of SAR11 having 15% accuracy (Morris et al. 2005).

Results

We designed experiments that enabled us to examine individual cultures of phytoplankton and bacterioplankton to determine if they could carry out VOC cycling functions. Few measurements of VOC metabolism by planktonic microorganisms have been made (Gantt et al. 2009; Hellebust 2000; Shaw et al. 2003; Sun et al. 2011), leaving it uncertain how much VOC cycling in the ocean surface is biological, which organisms participate, or how VOCs fit into metabolic schemes and genome predictions.

The study of VOCs by atmospheric chemists in recent years was advanced by the introduction of PTR-MS instruments, which are sensitive to VOC compounds that are otherwise difficult to measure at low concentrations (de Gouw and Warneke 2007). Figure 1 shows the experimental setup used to make the measurements reported in Figures 2 and 3. A continuous flow of carrier gas was introduced through a glass frit at the bottom of a vertical tube containing phytoplankton or bacterioplankton suspensions. Five tubes containing either cultures or control media with no cells could be incubated simultaneously in the presence of a controllable LED light source, under controlled temperature. The design was modified several times to achieve a

high flow of very small gas bubbles. The objective was to attain equilibrium between VOC concentration in suspension and VOC concentration in the gas phase entering the detector. The gas phase was fed directly into the PTR-MS, which measured VOC concentrations in real-time.

The diatom *Thalassiosira pseudonana* produced acetaldehyde at rates that were dependent on light intensity (Fig 2A). At the highest light intensity (1500 μmol photons m^{-2} s^{-1}), acetaldehyde production was approximately 160 μmol (g Chl*a* h)$^{-1}$. VOC production rates were fitted using the equation

$$VOC\ production\ rate = EF\ \times\ [ln(I)]^2$$

from Gantt et al. (2009) where EF is the emission factor (μmol VOC g Chl*a*)$^{-1}$ h^{-1}) and I is the light intensity cells were exposed to during an incubation (μmol photons m^{-2} s^{-1}). Acetaldehyde was below the limit of detection in incubations of growth medium alone and in actively growing *Dunaliella tertiolecta,* a green alga (data not shown). These controls obviate the possibility that acetaldehyde detection was released by the stripping chamber itself, or the inlet Teflon tubing connecting the dynamic stripping chambers to the PTR-MS.

T. pseudonana also produced isoprene and acetone in a light-dependent manner, although the rates were nearly 4-8 fold lower than acetaldehyde production (Fig 2B, C). A different profile of VOC production was observed in *D. tertiolecta. D. tertiolecta* produced methanol, isoprene, and acetone. Production rates for all three VOCs increased with light intensity (Fig 2D-F). In *D. tertiolecta* production rates were highest for methanol and acetone at the highest light intensity and averaged 40 μmol VOC (g Chl*a* h)$^{-1}$. This is the first direct measurement of a rate of methanol production by phytoplankton; the findings confirm that phytoplankton can be a source of methanol in ocean surface waters.

PTR-MS experiments showed that *Pelagibacter* strain HTCC7211 removed acetaldehyde from the suspension at the rate of 27 nmol (10^{10} cells h)$^{-1}$ when compared to control cultures treated with 1 mM sodium azide to block respiratory electron transport (Fig 3). The azide-treated control eliminated the possibility that acetaldehyde added to the culture was consumed by nonspecific reactivity. The cells used for these experiments were not treated in advance with acetaldehyde. In past work we found that most *Pelagibacter* carbon catabolism pathways are expressed constitutively and not induced by substrate addition (Sun et al. 2016).

Pelagibacter strains oxidize some VOCs without assimilating them into biomass (Sun et al. 2011). We confirmed acetaldehyde oxidation by two *Pelagibacter* strains (HTCC1062 and HTCC7211) and also studied its assimilation into biomass by feeding ^{14}C-labeled acetaldehyde to live cells. Scintillation counting was used to track the fate of the ^{14}C-label as it was transformed into $^{14}CO_2$ or cell biomass (Fig 4). The results demonstrated that both strains HTCC7211 and HTCC1062 oxidize acetaldehyde to CO_2. Furthermore, both strains incorporated about 30% of the utilized acetaldehyde into cell biomass. The rates of acetaldehyde utilization were in the same range as measured by PTR-MS, and were about twice as fast in HTCC7211 than HTCC1062. Rates of acetaldehyde incorporation and oxidation decreased after 3 - 8 h, which is typical of saturation. We do not know the cause of these apparent saturation kinetics for acetaldehyde, but in experiments with other substrates we have found that hours are required for intracellular concentrations to stabilize at their maxima (Sun et al. 2016). Alternatively, it is possible that a decrease in the cells' activities over time occurred, but this explanation seems less likely to us because many of *Pelagibacter's* metabolic pathways are constitutively expressed.

A pathway for aldehyde metabolism via the activity of the enzyme aldehyde dehydrogenase is common in bacteria (Sophos and Vasiliou 2003). The aldehyde dehydrogenase

(ALDH) superfamily comprises a group of divergently related enzymes that catalyze the oxidation of aldehydes. ALDHs in different subfamilies differ in their coenzymes and substrate specificities (Burton and Stadtman 1953). Figure 5 shows a hypothetical pathway for acetaldehyde metabolism in SAR11, which initiates with oxidation to acetate by the enzyme ALDH. Subsequently, acetaldehyde carbon can be either oxidized to CO_2 by entering the tricarboxylic acid (TCA) cycle, or it can be used to synthesize fatty acids.

We explored the genomes of *Pelagibacteraceae* for genes in the acetaldehyde dehydrogenase protein family that could explain the observation of acetaldehye oxidation by these strains, and studied the abundance of these genes in metagenomes (Fig 6). In total, 119 ALDH family genes were identified from HTCC1062, HTCC7211 and 17 other SAR11 strain genomes. In the HTCC1062 and HTCC7211 genomes, we identified a total of 15 genes in the acetaldehyde dehydrogenase family, and grouped them into eight ALDH-related subfamilies based on their protein sequence identities. Amino acid identity within the subfamilies was 60% or greater.

In the HTCC1062 and HTCC7211 genomes, two of the putative SAR11 ALDH genes (SAR11_0756 and HTCC7211_00006950) cluster with *E. coli aldB* genes from *Vibrio cholera, Escherichia coli* and *Ralstonia eutropha* with 62, 66, and 73% amino acid identities, respectively (Fig 6). In these bacteria, the *aldB* gene product has been demonstrated experimentally to function as an acetaldehyde dehydrogenase (Ho and Weiner 2005; Parsot and Mekalanos 1991; Priefert et al. 1992). Thus, we suggest that this SAR11 ALDH subfamily is most likely to be responsible for the acetaldehyde oxidation activity we observed in whole cells. This ALDH subfamily has a relative abundance of 1.38 (relative to the abundance of the single-copy *recA*

gene) in the GOS database, an indication it is common in the genomes of the SAR11 found in GOS samples.

Another SAR11 gene (SAR11_0796 and HTCC7211_00007350) shared 44% sequence identity with the *E. coli aldH* gene (Fig 6). The *E. coli aldH* gene product can also function as an aldehyde dehydrogenase, but it has relatively low activity with acetaldehyde substrate (Jo et al. 2008). Similarly, two putative SAR11 ALDH genes exhibit 46 and 40% sequence identities with rat methylmalonate-semialdehyde dehydrogenase (MMSDH) and succinate-semialdehyde dehydrogenase (SSADH), respectively (Fig 6). Both MMSDH and SSADH showed low activity with acetaldehyde (Goodwin et al. 1989; Rothacker et al. 2008). These SAR11 genes are abundant in the GOS database, but are less likely to be involved in acetaldehyde oxidation, as are the other ALDH subfamilies found in SAR11, which are more divergent (<40% sequence identity) from proteins with a demonstrated acetaldehyde dehydrogenase activity. SAR11 genes in the aldB and aldH subfamilies were mainly found in the genomes of the SAR11 IA ecotype, which are ocean surface strains (Fig 6B).

Discussion

Atmospheric chemists have led the way studying VOCs because of their importance to atmospheric processes. The oceans are estimated to emit ~22 Tg C y^{-1} to the atmosphere as VOCs (Gantt et al. 2009), but such estimates are limited by a lack of understanding about the biotic and abiotic sources and sinks of these compounds (Simó 2011; Williams et al. 2004). Air-sea fluxes quantify the transport of VOCs between these two large reservoirs and estimates of net emission rates vary markedly depending on the VOC species and ocean basin (Yang et al. 2014).

The challenge to quantify the magnitude of air-sea VOC flux requires an understanding of the sources and sinks, and the factors controlling these processes.

The biology of VOC cycling, for example knowledge about which organisms produce these compounds, under what circumstances they produce them, and what the metabolic roles of these compounds are, has barely been explored. To date, most attention has been given to understanding emission of dimethylsulfide into the atmosphere and its role in climate modulation. *T. pseudonana* produced acetaldehyde at rates that rival that of DMS production by diatoms in high light (Sunda et al. 2002; Sunda et al. 2007). This manuscript primarily focuses on acetaldehyde cycling, which, like DMS, is a climatically important VOC. Our results give the first rates of light dependent acetaldehyde production by phytoplankton, and show that bacterioplankton that are abundant in the ocean surface have the capacity to oxidize this compound to CO_2 and assimilate it into biomass. While these facts have been suspected (Dixon et al. 2013), this work demonstrates in a controlled setting, that plankton cells have acetaldehyde cycling functions and also constrains their rates of production and consumption.

The empirical measurements we made extrapolated to significant global rates of acetaldehyde cycling in the ocean euphotic zone (Fig 7). SAR11 cells are ubiquitously distributed in the oceans, where the SAR11 population in the global euphotic zone is estimated at 1.2×10^{28} cells (Morris et al. 2002). Assuming a constant rate of acetaldehyde production per unit chlorophyll, and 1.17×10^{12} g diatom chlorophyll in the global euphotic zone (see Methods), we estimated that the net capacity of the SAR11 community to oxidize acetaldehyde ($7.8 \times 10^{13} \pm 1.9 \times 10^{13}$ mol d^{-1}) considerably exceeds the calculated global production rate ($7.7 \times 10^{11} \pm 1.6 \times 10^{11}$ mol d^{-1}), implying that most acetaldehyde might be recycled before reaching the atmosphere. It is reasonable to expect that SAR11 has excess oxidative capacity for acetaldehyde

relative to its average rate of supply in the environment in order to gain the maximal advantage from harvesting a resource that fluctuates in its availability. This calculation provides useful first order constraints, but it is an approximation that does not take into account variation between diatom species, bacterioplankton other than SAR11, or abiotic processes, such as photooxidation of phytoplankton-derived CDOM, which can result in the production of acetaldehyde in the light (de Bruyn et al. 2011).

Given the observation that phytoplankton produce significant quantities of acetaldehyde, it is perhaps not surprising that aerobic bacterioplankton have found a way to harvest this labile DOM resource. Aldehydes are reactive compounds that form covalent adducts with nucleophilic residues, and are toxic to cells at high concentrations. As yet there is no information about acetaldehyde toxicity at the very low concentrations (4-37 nM) that cells encounter in the ocean environment. While the biochemistry of acetaldehyde production in phytoplankton is not known, the biochemistry of acetaldehyde oxidation is simple and would be easy to identify in genomes, were it not for the fact that the aldehyde dehydrogenase (ALDH) superfamily comprises a group of divergent but related enzymes that often are paralogs in genomes. ALDHs are $NAD(P)^+$-dependent enzymes which catalyze the oxidation (dehydrogenation) of a broad variety of aldehydes to their corresponding acids. In the simple genomes of SAR11 cells there are several enzymes classified as ALDH and it is not certain which is responsible for the observed acetaldehyde oxidation activity.

The acetaldehyde cycle is important because of the potential fates of acetaldehyde that enters the atmosphere and contributes to atmospheric chemistry, and also because acetaldehyde, as a volatile compound, is likely to be underestimated by methods routinely used for studying the carbon cycle (Suzuki et al. 1992). The acetaldehyde cycle is also a remarkably clear illustration

of the concept of the microbial loop, which describes the surprising reality that a significant quantity of organic carbon fixed by phytoplankton moves directly to bacterioplankton before entering trophic cascades. While there are a variety of mechanisms that collectively constitute the microbial loop, the example of acetaldehyde cycling clearly is a special case, where the volatility of the compound causes its loss from cells that produce it, and also makes it a favorable substrate for the smallest bacterioplankton, which rely on diffusion to obtain their carbon nutrients.

In addition to acetaldehyde, light-dependent isoprene production was detected in both of the phytoplankton species we tested (Fig. 2). Thus far, isoprene production appears to be widespread in open-ocean and coastal species (Bonsang et al. 2010; Colomb et al. 2008; Gantt et al. 2009; Shaw et al. 2003). The production rates we measured for *D. tertiolecta* and *T. weissflogii* were higher than those previously reported (Exton et al. 2013; Gantt et al. 2009), but these differences are likely due to culturing temperature (16 v. 20°C used in our study) and culture incubation methods for isoprene quantification (sealed vessels v. real-time, continuous measurement used in our study). In the field, isoprene concentrations are strongly correlated with chlorophyll concentrations (Broadgate et al. 1997; Orlikowska and Schulz-Bull 2009) reflecting cellular requirements for isoprene biosynthesis in actively growing cells.

The results we report demonstrate for the first time that phytoplankton produce methanol in the light, but not in the dark, which suggests that methanol is a product of photosynthetic metabolism (Fig. 2). Methanol has received attention recently because it is a significant component of the total VOC in the troposphere. Although seawater methanol concentrations are only 15-160 nM (Beale et al. 2015), it serves as an important growth substrate for a broad range of abundant bacterioplankton (Chistoserdova et al. 2009; Halsey et al. 2012; Morris et al. 2006;

Sun et al. 2011), raising questions about its sources in seawater. In this study, methanol production was detected in *D. tertiolecta* but not in *T. weissflogii.* Methanol was previously detected in the headspace of a range of taxonomically diverse phytoplankton monocultures including *T. weissflogii* (Riemer 1998). However, no previous studies have been published that have quantified light-dependent methanol production in real time.

Biosynthetic pathways for methanol have been investigated in land plants, but these studies have not yet been extended to phytoplankton. In land plants, methanol is released during demethylation of a pectin polysaccharide precursor (Fall and Benson 1996). Thus, methanol production is high in young, actively growing plants, and in leaves recovering from wounding (Hüve et al. 2007). Similarly, in phytoplankton, methanol production is suspected to be associated with cell growth since pectin is commonly found in phytoplankton species having cell walls, including *D. tertiolecta* (although in this species the pectin layer is very thin). The cell walls of diatoms are composed of silica and polysaccharides, but it is unclear whether pectins are components. Furthermore, genes encoding the pectin demethylase have not been detected in diatom or chlorophyta genomes (Wang et al. 2013). Nevertheless, these new data demonstrating algal production of methanol, combined with the well-established abundance of methylotrophs and methylovores in marine plankton communities (Halsey et al. 2012; Sun et al. 2011), suggest that biological methanol cycling directly impacts methanol emissions to the atmosphere. Clearly, more studies that investigate the specific sources and biochemistry of methanol production in seawater are warranted.

We also report direct measurements of light-dependent acetone production by two phytoplankton species (Fig. 2). Acetone production rates were very similar for both species. As with acetaldehyde, photodegradation of CDOM is thought to be the major source of acetone

emissions (Warneke et al. 1999; Zhou and Mopper 1997). Marine *Vibrio* species were also observed to produce acetone (Nemecek-Marshall et al. 1995). Thus, acetone production may have multiple biological sources. Acetone concentrations in seawater are very low (2-20 nM) and decreased during seasonal mixing in the Indian Ocean (Warneke and de Gouw 2001) and increased with eukaryotic phytoplankton cell densities (Beale et al. 2015). These observations suggest that phytoplankton are a source of acetone, either directly, or indirectly via photodegradation of organic carbon.

SAR11 cells, the most abundant group of DOM oxidizing cells in the oceans, are likely not the only acetaldehyde oxidizing microbial plankton, but our findings show that SAR11 populations alone are sufficient to explain previous reports of acetaldehyde cycling in the ocean surface (Dixon et al., 2013). This is particularly noteworthy given these cells have very small genomes, limiting the range of compounds they are able to oxidize. The implication is that acetaldehyde is a significant component of labile DOM. In the experiments described here acetaldehyde oxidation activity was expressed in SAR11 cells without induction, in agreement with other research indicating that these cells have limited transcription regulation, particularly of genes for carbon catabolism (Giovannoni et al. 2014; Sun et al. 2016)

For all the VOCs discussed here there is little known about the effects of environmental factors, such as nutrient, light, or trace metal availability, on VOC emissions. Future laboratory research that addresses these factors in concert with other photo-physiological characteristics that can be used to link rates of VOC production to remote sensing retrievals will lead to more accurate estimates of air-sea flux and how these emissions may, in turn, influence climate.

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Figure Legends

Figure 1. Schematic diagram of experimental apparatus consisting of environmentally controlled dynamic stripping chambers, from which a continuous stream of gasses emerges to enter a PTR-MS instrument, which measures targeted VOCs. The Dynamic Stripping Cell forces the liquid phase and gas phase into steady state. The rate at which the VOC is removed from the system corresponds directly to its partitioning coefficient.

Figure 2. Light-dependent production of VOCs by *T. pseudonana* (top) and *D. tertiolecta* (bottom). Exponentially growing cells grown at 60-70 mmol photons m^{-2} s^{-1} were transferred to dynamic stripping chambers, exposed to three or four light levels, and monitored for VOC production using a PTR-MS. Error bars are SE from exponential functions used to determine VOC production at each light level. See text for description of EF (emission factor) following Gantt et al. (2009). Light-dependent emission of acetaldehyde, isoprene, and acetone from *T. pseudonana* and methanol, isoprene, and acetone from *D. tertiolecta* were confirmed in two other independent experiments.

Figure 3. SAR11 bacterioplankton consume acetaldehyde. SAR11 strain HTCC7211 was harvested during exponential phase, concentrated by centrifugation and amended with 100 nM acetaldehyde in four individual dynamic stripping cells monitored by real-time PTR-MS. Top panel: Acetaldehyde was removed from duplicate chambers with live cells (red circles are averages of the duplicate measurements) at faster rates than duplicate chambers with cells treated with sodium azide (black circles are averages of the duplicate measurements). Similar results

were observed for a second independent experiment. Lower panel: the difference in loss rates due to HTCC7211 was calculated to be 36 ppbv h^{-1} (blue circles) or 27 nmol 10^{10} cells h^{-1}.

Figure 4. ^{14}C-labeled acetaldehyde utilization by SAR11 strains HTCC1062 (top panel) and HTCC7211 (bottom panel). Cells were harvested during exponential phase and resuspended in artificial seawater media (filled symbols). A portion of the suspension was treated with formalin and served as the killed control (open symbols). Suspensions were amended with100 nM ^{14}C-labeled acetaldehyde in sealed vials. Acetaldehyde incorporated into biomass (triangles) measured following addition of cold TCA and filtration. Acetaldehyde oxidized to $^{14}CO_2$ (squares) was determined by the difference of total acetaldehyde utilized (acetaldehyde oxidized + incorporated into biomass, measured following addition of base, Na_2CO_3, and $BaCl_2$ and filtration) and acetaldehyde incorporated into biomass. Bars are SD for triplicate measurements. Where not visible, error bars are smaller than the symbol.

Figure 5. A potential pathway of acetaldehyde metabolism in SAR11. Acetaldehyde is oxidized to acetate via aldehyde dehydrogenase. Following conversion of acetate to acetyl-CoA, acetaldehyde carbon either enters the TCA cycle or is used to synthesize fatty acids through lipogenesis.

Figure 6. Genomic data show that SAR11 genomes contain genes that are predicted to have aldehyde dehydrogenase activity. (A) Phylogeny of SAR11 genes in the aldehyde dehydrogenase (ALDH) family. Red labels at the branch tips indicate genes from SAR11 strains HTCC1062 and HTCC7211. Proteins with demonstrated acetaldehyde dehydrogenase activity

are labeled in blue. ALDH subfamilies are color coded by bars on the right in A and the left in B. (B) Relative abundance is the frequency of the gene in SAR11 genomes, as estimated from Global Ocean Survey metagenomic data. The categories, Ia.1, Ia.3, etc., refer to phylogenetic types that have distinct spatiotemporal or biogeographical distributions in the water column (Vergin et al., 2013). '+' indicates the presence of one ALDH subfamily gene, and '2' indicates the presence of two copies of ALDH genes in the genomes.

Figure 7. VOC cycling by marine plankton. A range of VOCs are produced by phytoplankton by light-dependent processes. The types of VOCs produced by phytoplankton are species-specific. Arrow thickness indicates relative rates of VOC production and consumption, and for acetaldehyde were 61 nmol $(10^{10}$ cells $h)^{-1}$ and 27 nmol $(10^{10}$ cells $h)^{-1}$, respectively (circled a and b). VOCs not consumed by bacterioplankton are free to escape to the atmosphere (orange circles). VOC utilization by bacterioplankton includes carbon assimilation into bacterial biomass and VOC oxidation to CO_2.

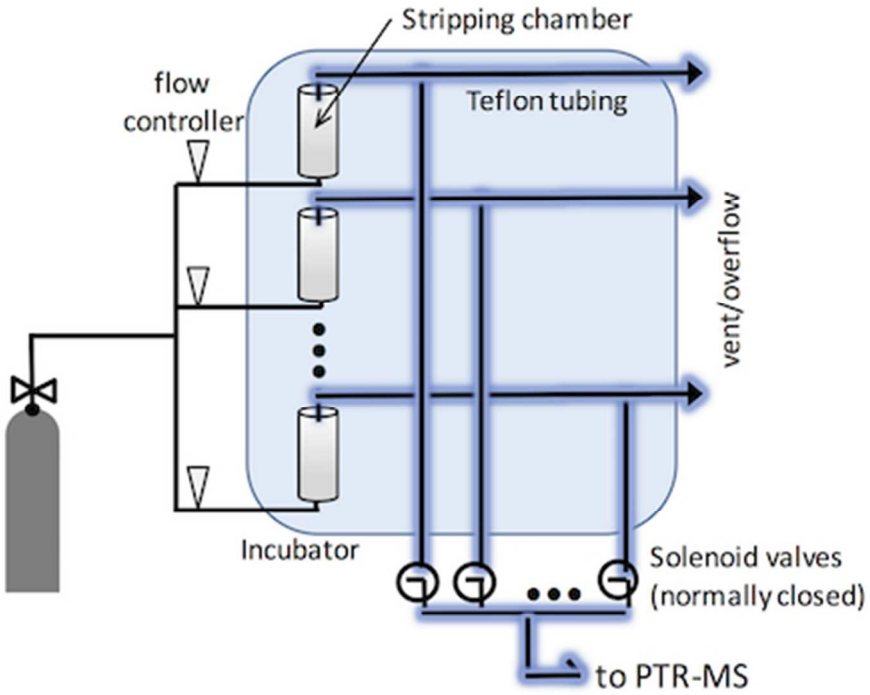

Figure 1. Schematic diagram of experimental apparatus consisting of environmentally controlled dynamic stripping chambers, from which a continuous stream of gasses emerges to enter a PTR-MS instrument, which measures targeted VOCs. The Dynamic Stripping Cell forces the liquid phase and gas phase into steady state. The rate at which the VOC is removed from the system corresponds directly to its partitioning coefficient.

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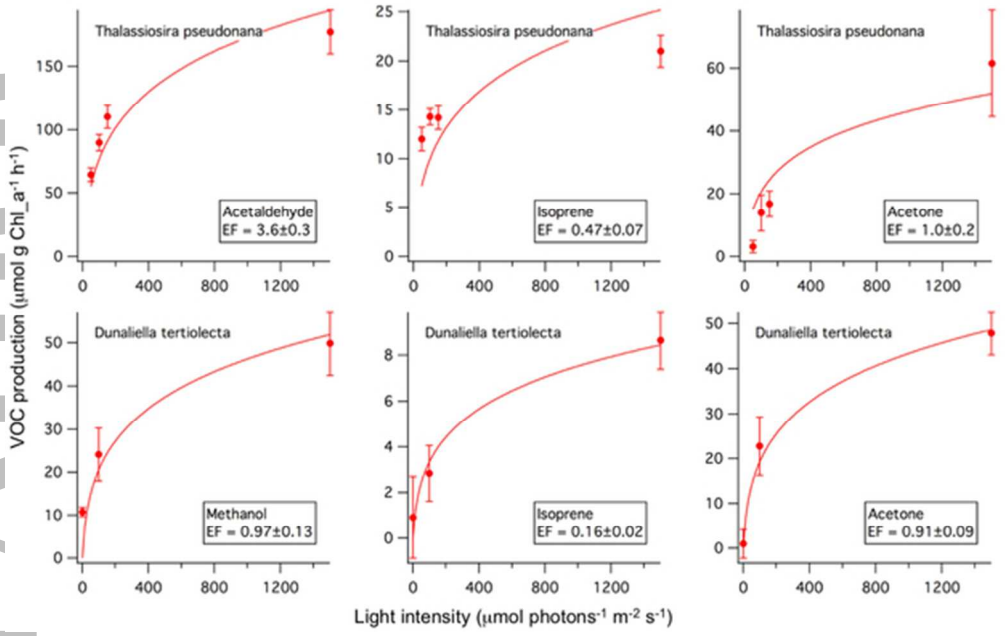

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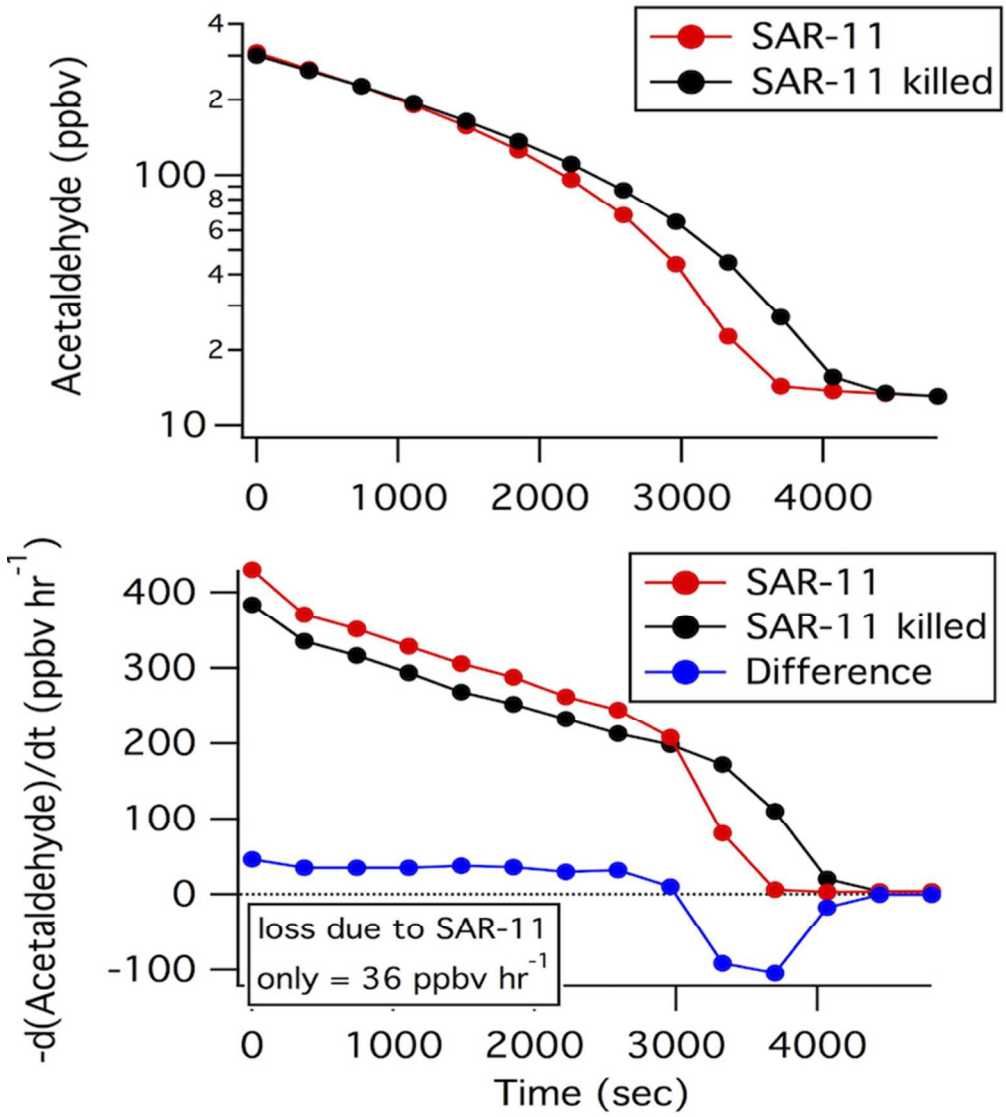

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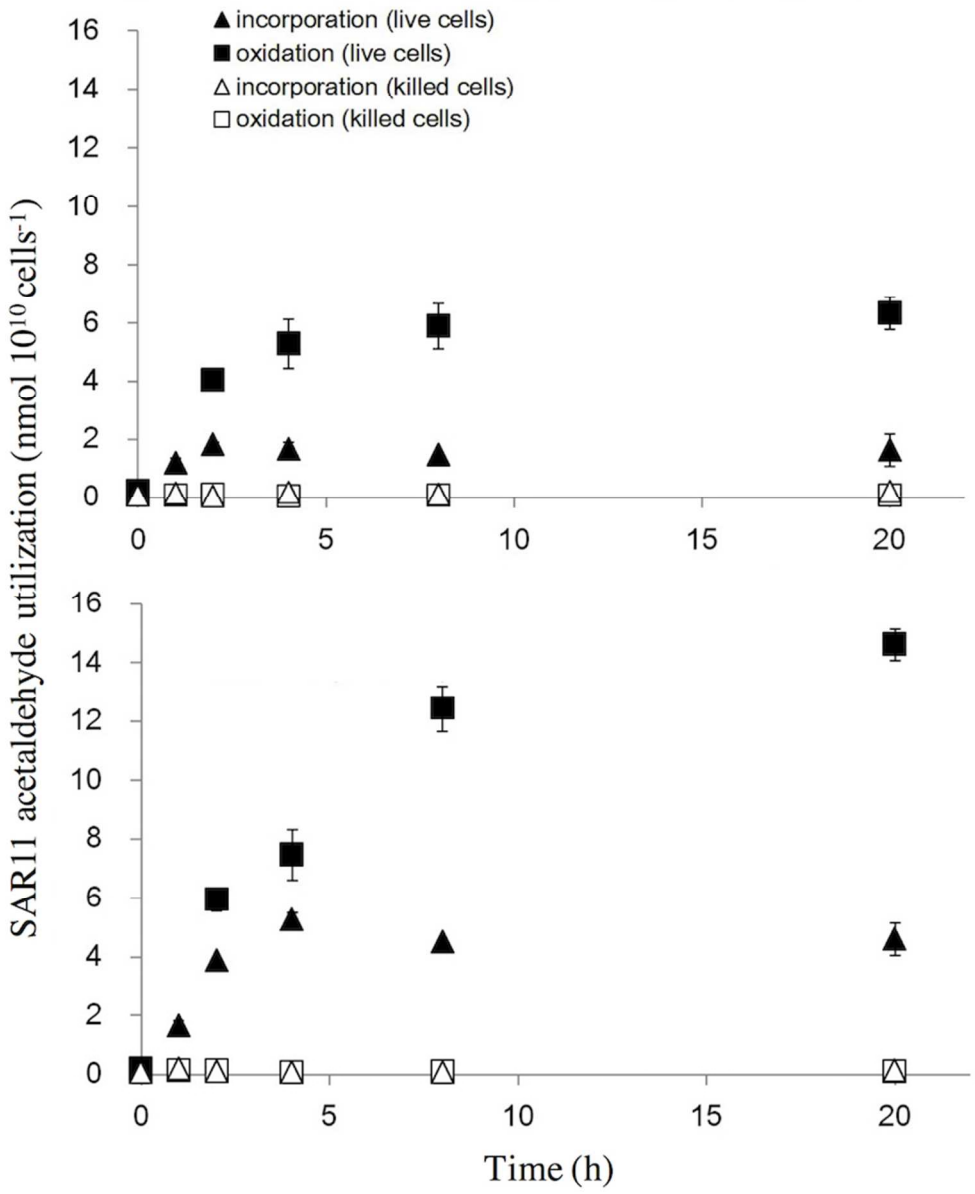

Figure 4. 14C-labeled acetaldehyde utilization by SAR11 strains HTCC1062 (top panel) and HTCC7211 (bottom panel). Cells were harvested during exponential phase and resuspended in artificial seawater media (filled symbols). A portion of the suspension was treated with formalin and served as the killed control (open symbols). Suspensions were amended with 100 nM 14C-labeled acetaldehyde in sealed vials. Acetaldehyde incorporated into biomass (triangles) measured following addition of cold TCA and filtration. Acetaldehyde oxidized to 14CO2 (squares) was determined by the difference of total acetaldehyde utilized (acetaldehyde oxidized + incorporated into biomass, measured following addition of base, Na2CO3, and BaCl2 and filtration) and acetaldehyde incorporated into biomass. Bars are SD for triplicate measurements. Where not visible, error bars are smaller than the symbol.

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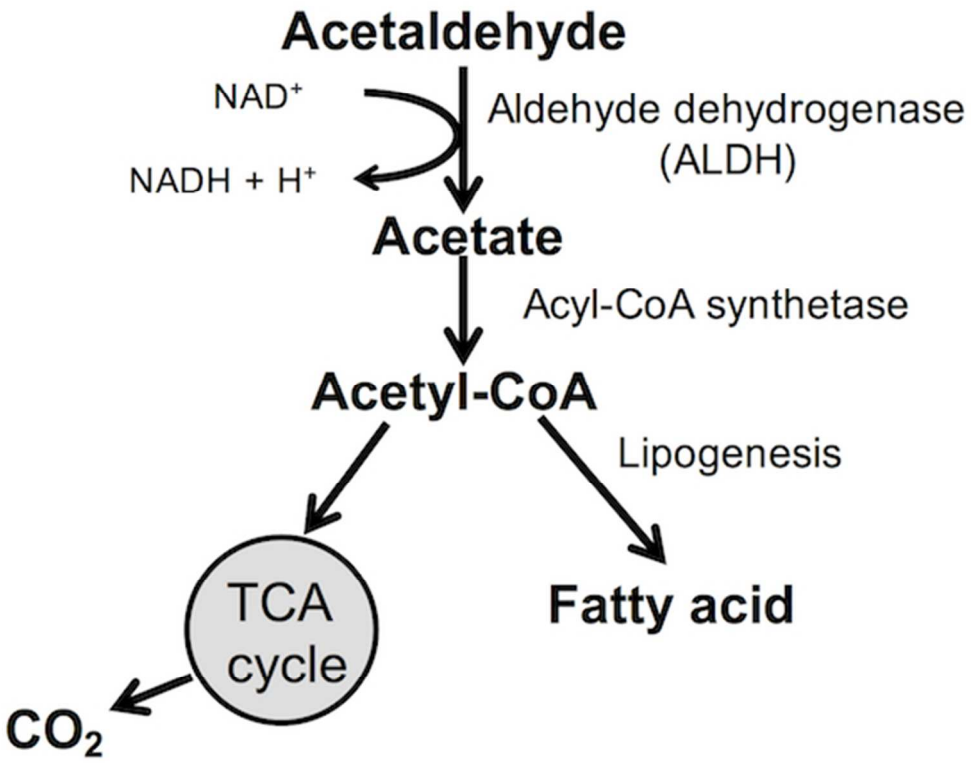

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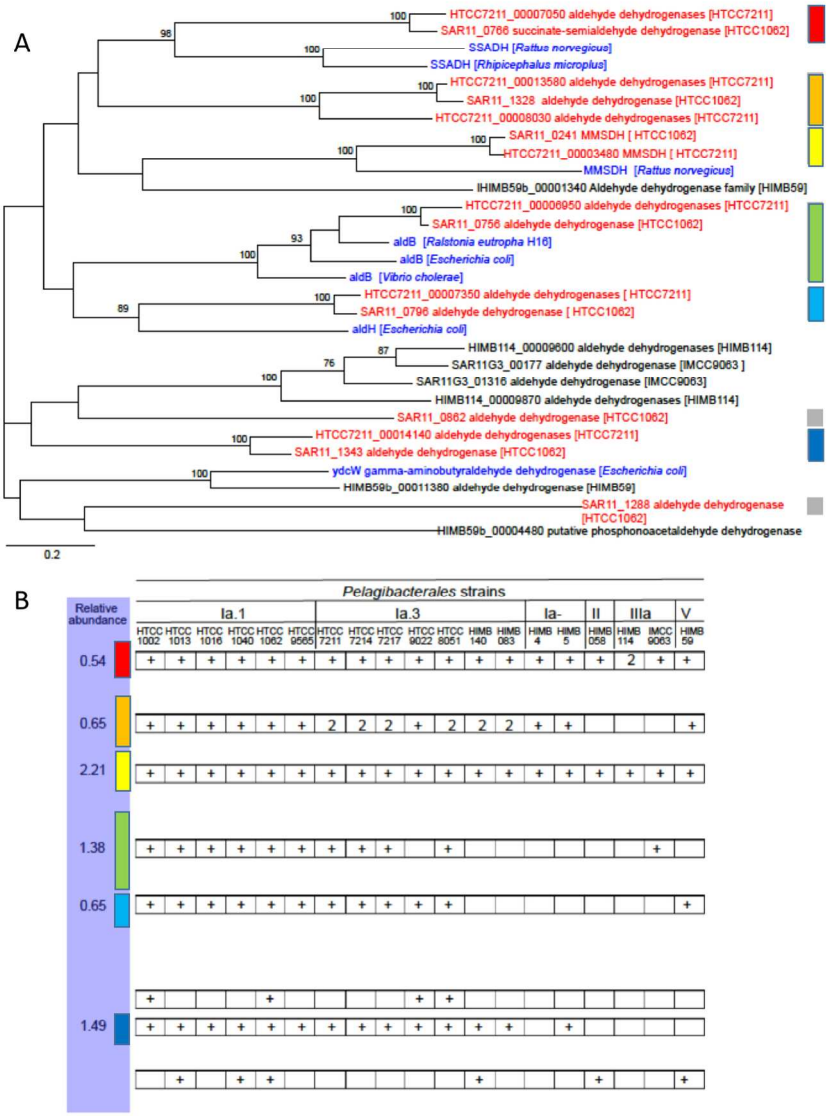
50x39mm (300 x 300 DPI)



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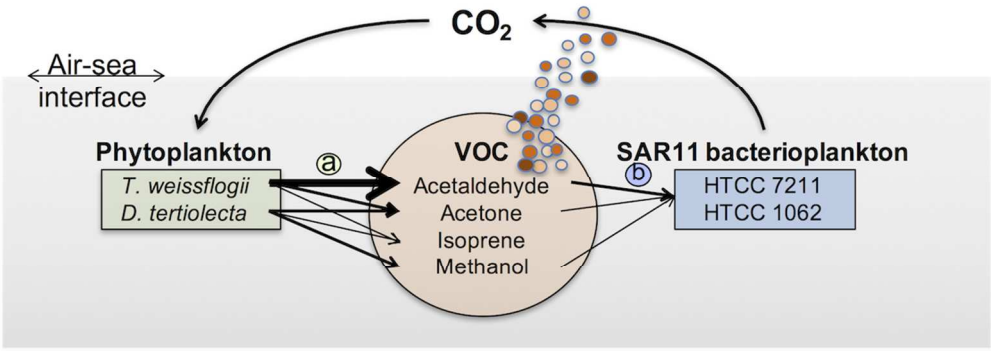

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110x39mm (300 x 300 DPI)