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Dissecting the role of viruses in marine nutrient cycling: bacterial uptake of D- and L-amino acids released by viral lysis

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ABSTRACT: Lysis of marine bacteria by viruses releases a range of organic compounds into the environment, including D- and L-amino acids, but the uptake of these compounds by other bacteria is not well characterized. This study determined that *Photobacterium* sp. strain SKA34 (Gammaproteobacteria) increased in abundance following uptake of D- and L-amino acids from viral lysate of *Cellulophaga* sp. strain MM#3 (Flavobacteria). Ammonium and dissolved free amino acids were taken up almost to detection limits, suggesting that the C:N ratio of bioavailable organic matter in the lysate was high for *Photobacterium* sp. growth, thus causing a net uptake of ammonium. In contrast, only 1.51 µmol l^{-1} of the 4.77 µmol l^{-1} of the total dissolved combined amino acids (DCAAs) were taken up, indicating that a fraction of lysate-derived DCAAs were semi-labile or refractory to bacterial uptake. Both D- and L-amino acid uptake rates were approximately proportional to their concentrations, indicating similar availability for each enantiomer and unsaturated uptake rates. These results imply that under high C:N conditions, both D-amino acids (mainly found in bacterial cell walls) and L-amino acids (found in proteins of the rest of the cell) are equally available for bacterial growth, and support arguments that viruses are key players in marine nitrogen cycling.

KEY WORDS: Viral lysis · Marine bacteria · Amino acid · Nitrogen · Uptake · Nutrient cycling · Ammonium

INTRODUCTION

A large portion of the total nitrogen (N) in the ocean consists of dissolved organic nitrogen (DON), a fraction of which includes cellular components such as proteins, nucleic acids, metabolites, and cell wall substances, as well as transformed organic matter in humic substances (McCarthy et al. 1997, Berman & Bronk 2003). Among components in the DON pool, smaller nitrogenous molecules such as free and combined amino acids and urea are important in the cycling of N since they are relatively labile and can be assimilated by microorganisms (Antia et al. 1991).

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Measurable concentrations of amino acids (AAs) in the surface ocean typically range from 0.3 to 1.0 µmol l^{-1}, but the total concentrations are likely higher due to inefficient methods for detection of all AAs and because many AAs are in the form of hydrolysis-resistant amide groups (Keil & Kirchman 1993, McCarthy et al. 1996). Most of the N in living organisms is contained in AAs (McCarthy et al. 1998), including both dissolved free and combined AAs (DFAAs and DCAAs). Although most AAs within organisms are homochiral with the L-AA enantiomer dominating (Bonner 1995), there is a significant pool of D-AAs in the ocean (McCarthy et al. 1998). D-AA isomers are a component of the peptidoglycan in bacterial cell walls (McCarthy et al. 1998), and can also be found in other parts of bacteria such as in lipopeptides (Kaiser & Benner 2008). D-AA measurements in sea water indicate that a substantial portion of AAs in DON originates from bacteria (McCarthy et al. 1998), and D-AA enrichment in refractory DON pools indicates that bacterial cell walls are more refractory than proteins (Berman & Bronk 2003, Nagata et al. 2003, Kitayama et al. 2007).

In addition to cell wall material release, DON in the ocean is produced by phytoplankton (e.g. Sarmento et al. 2013) and bacteria (e.g. Kaiser & Benner 2008), as well as from viral lysis of phytoplankton (Gobler et al. 1997, Hewson et al. 2004, Haaber & Middelboe 2009) and bacteria (Middelboe et al. 2003, Middelboe & Jørgensen 2006). Each day, 10 to 20% of bacteria in the ocean are killed by viruses (Suttle 1994). Lysis of infected bacteria releases DON, including DCAAs, DFAAs, and other cellular components (Middelboe & Jørgensen 2006). This influx of organic matter enhances bacterial production and fuels the microbial loop and the cycling of inorganic nutrients (Fuhrman 1999, Wilhelm & Suttle 1999, Middelboe & Lyck 2002). AAs are taken up via a variety of transport systems that vary in specificity (Crawford et al. 1974, Antia et al. 1991, Montuelle et al. 1992). In eukaryotes, the uptake is often stereospecific with different transport systems for D-AAs and L-AAs (Suzuki 1981, McDaniel et al. 1982). Similar uptake systems may occur in bacteria, since uptake and release of D-AAs can be involved in rearrangements of peptidoglycan during specific growth phases (Cava et al. 2011, Azúa et al. 2014), and since uptake of D-AAs is stimulated during low-nutrient conditions in marine bacteria (Pérez et al. 2003). Since bacteria constitute a large portion of the living biomass in the sea (Whitman et al. 1998), release and viral lysis of bacterial cells, including the release of AA isomers from cell wall material, are assumed to contribute significantly to the N cycle in the ocean and possibly to the production of refractory organic matter (Jiao et al. 2010). Consequently, knowing the fate of labile N substances from bacteria is relevant to understanding marine N cycling.

Here, we examined whether the lysis products of one bacterial species can support growth of another species. Previous studies have shown that compounds released by viral lysis of the Flavobacteria *Cellulophaga* sp. can be taken up by uninfected bacteria (Middelboe et al. 2003, Middelboe & Jørgensen 2006), but these studies did not examine uptake of the specific compounds of viral lysis by other bacterial taxa. In this study, we monitored the production of ammonium, DFAAs, and DCAAs during viral lysis of the marine heterotrophic bacterium *Cellulophaga* sp. strain MM#3, and its subsequent uptake of N compounds, including D- and L-AAs, by another marine bacterium, *Photobacterium* sp. strain SKA34. These experiments help us understand the central role that viruses may play in nutrient cycling in the marine environment.

MATERIALS AND METHODS

Growth of *Cellulophaga* sp. and lysate preparation

Cellulophaga sp. strain MM#3 was grown in 70% 0.2 µm filtered sea water and 30% ultrapure water containing 0.25 mmol l^{-1} lactose as a carbon source, 0.07 mmol l^{-1} K_2HPO_4, and 0.26 mmol l^{-1} $NaNO_3$ (modified from Middelboe et al. 2003). The strain belongs to a group of *Bacteroidetes* strains that have been isolated from 5 different locations in Danish and Swedish coastal waters during a 10 yr period (Holmfeldt et al. 2007), and thus are considered a prevalent group in this environment. Cultures were grown on the bench top at room temperature. Lysates of *Cellulophaga* sp. previously infected with a *Cellulophaga*-specific virus (Cellulophaga P1; Middelboe et al. 2003) were spun down at 37 000 × *g* for 1 h and resuspended twice in SM buffer (100 mmol l^{-1} NaCl, 17 mmol l^{-1} $MgSO_4 \cdot 7H_2O$, 50 mmol l^{-1} Tris-HCl pH 7.8) to remove cellular debris. At approximately 4.0×10^7 cells ml^{-1}, the virus was added at a multiplicity of infection of 5, i.e. 20×10^7 viruses ml^{-1}. Bacterial abundance was measured by flow cytometry as described below, and the concentration of infectious viruses was determined by plaque assay at 0, 21.25, and 28.25 h. Plaque assays were performed by combining a series of stock *Cellulophaga* sp. cultures with increasingly diluted experimental samples,

adding these mixtures to agar plates, and counting the plaques (areas of plate that did not form a bacterial lawn). When viral abundance had increased 200-fold, the culture was terminated by sequential filtration through glass-fiber GF/F and 0.2 µm pore size Cyclopore filters (Whatman). The filtered sample thus represented the dissolved fraction of the virus-derived organic and inorganic matter following cell lysis, as some of the lysate (e.g. larger cell fragments) may have been retained on the 0.2 µm filter; in addition, any of the media components that were not taken up by the *Cellulophaga* (lactose, $NaNO_3$, K_2HPO_4) may have been present. Samples for bacterial abundance, ammonium, DFAAs, and DCAAs were taken at 0 and 28.25 h.

Addition and uptake of lysate

With the exception of control cultures, 100 ml of filtered lysate was added to 400 ml triplicate cultures of the marine *Photobacterium* sp. strain SKA34 (initial cell abundance 16.0×10^6 cells ml^{-1}) grown in a nutrient-rich medium and washed via centrifugation and inoculated in artificial sea water at a salinity of 15, without added nitrate or phosphate (Harrison et al. 1980, modified as in Cottrell & Suttle 1993). Like *Cellulophaga* MM#3, this strain was also isolated from coastal Danish waters (Pinhassi et al. 2003). *Photobacterium* sp. is a member of the Gammaproteobacteria, a class that is often important in dissolved organic matter assimilation (Elifantz et al. 2007), and can dominate bacterial communities exposed to dissolved organic matter from diatoms (Landa et al. 2013). Because *Photobacterium* sp. SKA34 cannot use lactose as a carbon source (Middelboe et al. 2003), any growth or respiration with the addition of filtered lysate would not be from residual lactose in the *Cellulophaga* sp. growth medium. Cultures were kept in the dark at 10°C. Samples for bacterial abundance, and ammonium, DFAA, and DCAA concentrations were taken approximately every 6 h for 69.5 h. Triplicate control cultures without lysate addition were established in parallel.

Treatment and analysis of samples

Samples for bacterial abundance were fixed with gluteraldehyde at a final concentration of 0.5%, flash frozen in liquid nitrogen, and stored at −80°C until analysis. Bacterial abundance was measured using a flow cytometer (BD FACSCanto™ II, Beckton Dickinson) following Brussaard (2004). Samples for ammonium, DFAA, and DCAA determination were filtered through glass fiber filters (Whatman) into 15 ml polycarbonate tubes, and frozen at −20°C until analysis. Ammonium from all 3 replicates was determined from the filtered samples with a fluorometer (Holmes et al. 1999).

Dissolved free and combined amino acids (DFAAs and DCAAs) in two of the replicate cultures (#1 and #2) were quantified by high-performance liquid chromatography (HPLC) and fluorescence detection using 2 methods. Total amounts of DCAAs (after hydrolysis, see below) and DFAAs were detected as fluorescent primary amines after derivatization with *o*-phthaldialdehyde (OPA) according to Lindroth & Mopper (1979) and Jørgensen et al. (1993). For the analysis of DCAAs, triplicate water samples were freeze dried and subsequently hydrolysed by a microwave technique (Jørgensen & Jensen 1997). The hydrolysed samples were re-dissolved in 1.25 mol l^{-1} borate buffer at pH 9.5 and analysed by HPLC. Concentrations of DFAA were subtracted from the concentrations of total dissolved hydrolysable AAs to provide DCAA concentrations. The D-and L-isomers of Asp, Glu, Ser, and Ala were detected according to Mopper & Furton (1999) with the exception that N-isobutyryl-L-cysteine (IBC) was used as a chiral agent (Brückner et al. 1994). Analysis of D-AA isomers focused on Asp, Glu, Ser, and Ala as these AAs have been found to be dominant isomers in natural and biological material, including in bacterial peptidoglycan (Brückner et al. 1994, Jørgensen et al. 2003). HPLC columns were 3.9 µm, 150 mm steel columns, type Nova-Pak C18 (for the OPA method) and XTerra RP18 (for the IBC method) (Waters Associates). Analytical variation of DFAA concentrations between replicate analyses (injections) was below 5%.

RESULTS

Uptake of nitrogen from lysate by *Photobacterium* sp.

The abundance of *Photobacterium* sp. cells in the cultures enriched with lysate increased from an initial value of 2.64×10^5 cells ml^{-1} to 2.14×10^7 cells ml^{-1} at 69.5 h (Fig. 1) based on the uptake of viral lysates produced from infection of *Cellulophaga* sp. In the control cultures, no growth was found, and the cell abundance declined from 2.16×10^6 cells ml^{-1} at start of the incubation to 1.05×10^6 cells ml^{-1} at 69.5 h.

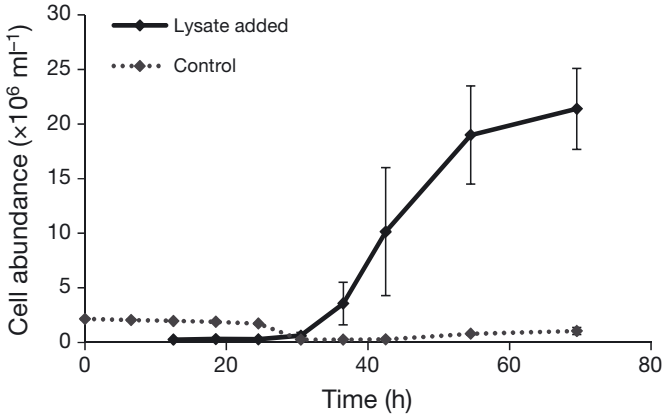

Fig. 1. Changes in *Photobacterium* sp. abundance over time following addition of lysate and in the control culture. Error bars are standard deviations from triplicate incubations

Concentrations of nitrogenous compounds in the lysate-enriched cultures showed an increased uptake of both ammonium and AAs relative to the controls during the incubation period (see Fig. 3). The concentration of ammonium declined from an initial value of 2.3 µmol l^{-1} to <0.1 µmol l^{-1} in the lysate-enriched cultures, with a reduction from 0.39 to 0.32 µmol l^{-1} in the controls (Fig. 2A).

For DFAA, the concentrations increased from 0.291 to 0.407 µmol l^{-1} within the initial 24.5 h, before a rapid decline to 0.036 µmol l^{-1} at the end of the incubation (Fig. 2B). In the controls, DFAA fluctuated from 0.043 to 0.055 µmol l^{-1} and no trends were found. Relative to all DFAA, concentrations of the 4 measured D-isomer DFAAs (D-Asp, D-Glu, D-Ser, and D-Ala) varied from 0.0032 to 0.0076 µmol l^{-1}, corresponding to 1 to 2% (initial 24.5 h) and 4 to 6% (30.5 to 69.5 h) of all DFAA. In the lysate-enriched cultures, the D-DFAA concentrations increased during the initial 24.5 h followed by a gradual decrease (Fig. 2B). In the controls, the D-DFAAs varied between 0.0023 and 0.0035 µmol l^{-1}, corresponding to about 50% of the concentrations in the lysate-enriched cultures (Fig. 2B). The D-DFAAs were dominated by D-Ala and D-Glu, making up 62 and 21% of all the D-DFAAs, respectively. The D-DFAA composition in the controls differed from the lysate-enriched cultures and was dominated by D-Asp and D-Ala, constituting 43 and 29% of the D-DFAAs, respectively.

Concentrations of DCAA varied slightly between cultures #1 and #2, but the net uptake during the incubation period was similar in both cultures (Fig. 2C). DCAA concentrations declined from 5.55 and 3.99 µmol l^{-1} at the start to final concentrations of 3.89 and 2.64 µmol l^{-1} for cultures #1 and #2, respectively (Fig. 2C). The declines corresponded to a DCAA uptake of 1.35 (#1) and 1.66 (#2) µmol l^{-1} during the 69.5 h incubation. The reason for the initial difference in DCAAs between the 2 cultures is unknown, but similar changes after 24.5 h associated with the increase in bacterial abundance suggested that 30 to 35% of the DCAAs in the lysates was

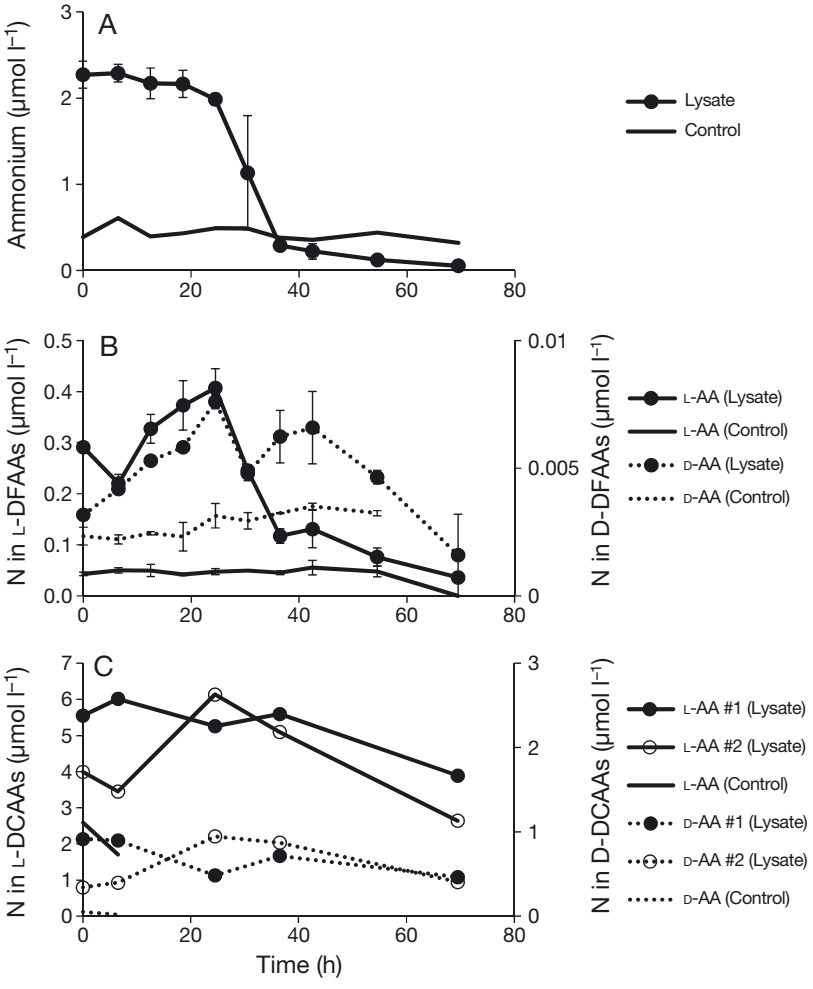

Fig. 2. Uptake of nitrogenous compounds by *Photobacterium* sp. over time: (A) ammonium, (B) dissolved free amino acids (DFAAs) and their D-AA isomers, and (C) dissolved combined amino acids (DCAAs) and their D-AA isomers. Error bars in (A) are standard deviation from triplicate incubations. The bars in (B) are the range from duplicate incubations. In (C), replicates are shown separately

utilized by *Photobacterium* sp. during the incubation. In the controls, mean DCAA concentrations were reduced from 1.1 to <0.7 µmol l^{-1} during the incubation (Fig. 2C). Dominant individual DCAAs in the lysate-enriched cultures were L-Glu, L-Asp, L-Ala, and Gly, together constituting 43 to 56% of all DCAAs. At end of the 69.5 h incubation period, the proportion of these DCAAs was reduced and made up 38 to 45% of the DCAAs (data not shown).

D-isomer DCAAs (D-DCAAs) constituted 10 to 18% of the total initial DCAA pool. A fraction of the D-DCAAs in the lysate was available for bacterial uptake as indicated by the decline in D-DCAA concentrations from maxima of 0.98 (#1) and 1.01 µmol l^{-1} (#2) to about 0.50 µmol^{-1} at 69.5 h (Fig. 2C). No significant change was observed in the relative contribution of D-DCAAs during the incubation. The D-DCAAs were dominated by D-Asp (53% mean value) with equal proportions of D-Glu, D-Ser, and D-Ala contributing to the remainder.

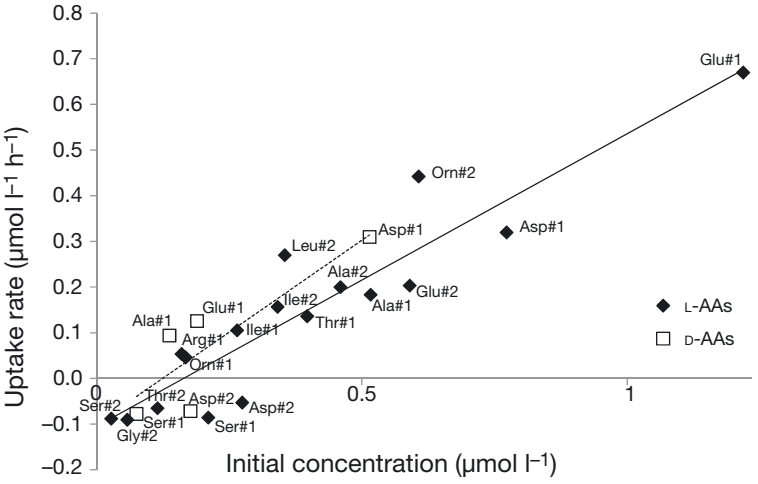

Fig. 3. Uptake of specific L- and D-isomers of dissolved combined amino acids by *Photobacterium* sp. for each amino acid as a function of its initial concentration from replicates #1 and #2. Uptake rate is calculated by subtracting the final concentration from the initial concentration for each amino acid. Linear regression is significant for L-amino acids (L-AAs) (solid line; $p < 0.001$, $R^2 = 0.86$) and not significant for D-amino acids (D-AAs) (dotted line; $R^2 = 0.74$)

To determine relations between concentrations of DCAAs and their uptake rates, the measured rates were plotted against initial concentrations of the individual DCAAs (Fig. 3). The figure indicates that uptake was concentration dependent, at least for the most abundant DCAAs such as L-Glu, L-Asp, D-Asp, L-Ala, and Thr. Analysis of the change in concentrations of individual AAs showed a net decrease in most AAs, with the largest uptake found for L-Glu, L-Asp, and L-Orn (Fig. 3). Negative uptake rates (e.g. Gly#2, Ser#1) indicate that there was a net release of some AAs. One AA, serine, was released as both L- and D-isomers, indicating accumulation of Ser-rich material in the water during the incubation. A comparison of uptake rates of L- and D-isomers among the DCAAs shows that L-Glu was taken up 5-fold faster and L-Ala was taken up 2-fold faster than the corresponding D-isomers in replicate #1, while similar rates were found for uptake of L- and D-Asp and for release of L- and D-Ser (data not shown).

DISCUSSION

This study demonstrates that the marine bacterium *Photobacterium* sp. strain BKA34 takes up D- and L-AAs released by viral lysis of *Cellulophaga* sp. strain MM#3 in proportion to their relative abundance; however, only about one-third of the AA content of the dissolved lysates was immediately available for bacterial uptake and there are differences in the relative uptake among AAs. These observations are important for understanding the fate of the products of viral lysis in the sea.

Bioavailability of amino acid nitrogen in viral lysates and their contribution to bacterial nitrogen demand

Bacterial lysates are generally assumed to contain excess N relative to bacterial N demand due to the low (4 to 5) C:N ratio of bacterial cells and bacterial respiratory loss of >70% of the carbon (C) uptake (Middelboe et al. 1996). Consequently, bacterial uptake of lysates is expected to result in net remineralisation of N. Instead of the expected ammonium remineralisation (Hollibaugh 1978, Shelford et al. 2012), ammonium and DFAAs were taken up rapidly (Fig. 2A,B), indicating that initial concentrations of organic C relative to N were in excess of the growth needs of *Photobacterium* sp. Assuming a cell C content of 20 fg cell^{-1} (Carlsson et al. 1999) and a C:N ratio of 4.5 (Goldman et al. 1987) for *Photobacterium* sp., the estimated bacterial N uptake was 16 ± 4 µmol l^{-1} N during the 69.5 h incubation. Of this N demand, bacterial growth was supported by uptake of 0.3, 1.5, and 2.3 µmol l^{-1} of DFAAs, DCAAs, and ammonium, respectively, corresponding to 25% of the estimated

total bacterial N demand. This suggests that available N sources other than AAs were released from viral lysis and emphasises that the initial C:N ratio of bioavailable organic matter in the lysate-enriched cultures was higher than required to allow mineralization of excess N.

The decrease in bacterial growth rate (i.e. stationary phase) after ~40 h (Fig. 1) combined with a relatively slow pull down in DCAA (Fig. 2C) and no remineralisation of N indicates a continuing shortage of N. Despite conditions where the DFAA pool was exhausted and bacterial growth was most likely substrate limited, 68% of the DCAA pool was still not used by the bacteria. This suggests that a significant portion of the DCAAs were not readily available for uptake and growth by *Photobacterium* sp. This may be because a portion of the DCAAs had yet to be degraded, possibly by enzymatic activity, into a form that bacteria could take up, thus resulting in a delay in the DCAA uptake (Nagata et al. 1998, Murray et al. 2007). However, DCAAs released by viral lysis may also potentially be a source of refractory DON, as DCAAs derived from bacterial membranes have been shown to persist and accumulate in the ocean (Tanoue et al. 1995). More recently, the presence of viruses has been shown to increase the formation of humic-like dissolved organic matter by a factor of 2.8 compared with incubations without viruses (Lønborg et al. 2013), further supporting that viral lysis may contribute to the formation of refractory DON.

Marine bacteria grow at different rates and vary in their gross growth efficiency when grown on free AAs with different C:N ratios (Goldman et al. 1987, Goldman & Dennett 1991). Yet, despite an apparent shortage of N for growth, the current study saw no evidence for a preference for low C:N ratio AAs among DCAAs. This indicates that the uptake of DCAAs is not simply governed by the C:N ratio of the DCAA substrate, but may be dependent on other factors, such as the presence of specific transport mechanisms (Razin et al. 1968, Cheruel & Jullien 1979, Montuelle et al. 1992), the need for specific AAs in metabolic reactions, the requirement for enzymatic degradation (Nagata et al. 1998, Murray et al. 2007), or total AA concentration.

Uptake of different AAs by *Photobacterium* sp.

The differences in uptake among AAs were likely the result of initial concentrations, as seen from the linear relationship between uptake rate and initial concentration (Fig. 3), indicating that uptake rates were not saturated and maximum uptake rates were not reached. Uptake kinetics are dependent on the nutritional status of the bacteria (Jørgensen 1992), and different uptake systems can be present at different substrate concentrations (Unanue et al. 1999). In the current study, the AAs with the highest concentrations had the highest uptake rates, indicating that *Photobacterium* sp. took up AAs based on their relative availability.

Unlike the other DCAAs, there was production of L-Ser in both replicates and D-Ser in replicate #1 (Fig. 3). Serine is often a dominant DFAA in natural waters (e.g. Thurman 1985, Hubberten et al. 1995), but can sometimes be unavailable for bacteria and phytoplankton due to restricted uptake capacity (Palenik & Morel 1990, Ietswaart et al. 1994). This may explain the presence of Ser in natural waters. We speculate that in the present study, the accumulation of L- and D-Ser in the DCAA pool at 69.5 h may reflect release of peptidoglycan components due to cell wall transformations in the stationary growth phase, as observed for D-Ala in a *Vibrio* species by Azúa et al. (2014). Both isomers of Ser have previously been found in peptidoglycan (Jørgensen et al. 2003, Pérez et al. 2003). The dominance of different isomers among D-DCAAs (D-Asp made up 53%) and D-DFAAs (D-Ala made up 62%) in the present cultures suggests that the dynamics of production and uptake of individual amino acids are different in the 2 AA pools. In axenic cultures of 2 *Vibrio* species, Azúa et al. (2014) found that D-DCAAs largely consisted of D-Ala and D-Asp. The low occurrence of D-Ala (15% of the D-DCAAs) and also the presence of D-Glu and D-Ser in our cultures demonstrate that the source and degradation patterns of D-AAs are variable, as further discussed below. More studies are needed to document relations between specific extra- and intracellular D-isomers, cell growth phases, and bacterial species.

Availability of both AA enantiomers

Measurements of both D- and L-AAs indicated each AA uptake rate by *Photobacterium* sp. was proportional to its initial concentration (Fig. 3). The main sources of D-AAs are bacterial cell wall components including peptidoglycans, lipopolysaccharides, and lipopeptides (Kaiser & Benner 2008). It is generally assumed that D-AAs and bacterial cell wall material are somewhat refractory (Grutters et al. 2002, Nagata et al. 2003, Kitayama et al. 2007) and can accumulate in sea water (Dittmar et al. 2001), compared with more labile proteins originating from membranes

and intracellular material. The D-enantiomer of amino acids can be used as a prokaryotic biomarker, since prokaryotes are the main producers of D-AAs in the ocean (Kaiser & Benner 2008). In contrast, phytoplankton activity supplies mainly L-AAs, which have been shown to be taken up preferentially over D-AAs (Pérez et al. 2003). However, there is evidence that D-AA uptake increases in the low-nutrient deep ocean (Pérez et al. 2003), that it depends on whether the source is from Gram-negative or Gram-positive bacterial cell walls (Jørgensen et al. 2003), and that uptake rates of certain free D-AAs increase with concentration (Azúa et al. 2014). Data from the current study suggests that D-AAs (from *Cellulophaga* cell walls) and L-AAs (likely from intracellular and membrane sources) are taken up in proportion to their initial concentrations. For example, the 7-fold greater uptake of L-Glu relative to D-Glu was consistent with its higher initial concentration, while the concentrations and uptake rates of L-Asp and D-Asp were similar (Fig. 3). In microcosms with marine bacterioplankton, Azúa et al. (2014) observed comparable uptake of free L- and D-Ala, but a reduced uptake of D-Asp relative to L-Asp. Here we observed similar uptake rates of combined L- and D-Asp, but reduced uptake rates of free D-Ala and D-Asp relative to the L-isomers, possibly indicating that different uptake mechanisms exist for combined and free AAs.

The results presented here indicate that AAs in viral lysates from *Cellulophaga* sp. are taken up by *Photobacterium* sp. Both bacterial species are Gram-negative bacteria, and since Gram-negative bacteria dominate the world's oceans, this system is likely a reasonable model for the fate of AAs from the lysis of most marine bacteria. However, Gram-positive bacteria are diverse and potentially more abundant than previously considered (Gontang et al. 2007), as well as being biologically very different from Gram-negative bacteria (Gupta 2000). A previous study suggests that peptidoglycan of Gram-positive species is more labile than that of Gram-negative species (Jørgensen et al. 2003), and therefore, efforts should be undertaken to examine the fate of AAs released by viral lysis of Gram-positive bacteria as well, to gather a more comprehensive picture of the role of viral lysis on nutrient cycling in the ocean.

In addition, the use of a single strain of bacteria limits generalisation. Under natural conditions with a complex bacterial community that specialises in uptake of different nitrogenous products, it may be that the results are different from those found here. However, this study introduces data that warrant further exploration.

Conclusions and ecological implications

There are a few surprising observations from the study presented here. First, the uptake of AAs was not associated with the remineralisation of ammonium that has been observed in many studies (e.g. Hollibaugh 1978, Goldman et al. 1987, Shelford et al. 2012). Second, only about one-third of the DCAAs in the viral lysate was taken up over the 5 d of this study, indicating that many of the DCAAs released by viral lysis were not immediately available for uptake. Third, there was no evident discrimination in uptake of the D-AAs associated with bacterial cell walls relative to L-AAs associated with bacterial proteins, in contrast to results from previous studies (Kawasaki & Benner 2006, Azúa et al. 2014). In contrast to the previous studies that have suggested bacterial utilization of viral lysates as a potentially important pathway for N mineralization supporting phytoplankton growth (Weinbauer et al. 2011, Shelford et al. 2012), the current study emphasizes that the metabolism of viral lysates may result in net consumption of inorganic N. Because an estimated 10 to 20% of marine bacteria are lysed by viruses every day (Suttle 1994), and bacteria represent most of the biomass in the oceans, it is crucial to determine the pathways and fate of nutrients released by viral lysis.

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