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Uptake of Dissolved Organic Carbon by Gammaproteobacterial Subgroups in Coastal Waters of the West Antarctic Peninsula

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Heterotrophic bacteria are well known to be key players in the turnover of dissolved organic material (DOM) in the oceans, but the relationship between DOM uptake and bacterial clades is still not well understood. Here we explore the turnover and single-cell use of glucose, an amino acid mixture, *N*-acetylglucosamine (NAG), and protein by gammaproteobacterial clades in coastal waters of the West Antarctic Peninsula in summer and fall. More than 60% of the cells within two closely related gammaproteobacterial clades, Ant4D3 and Arctic96B-16, were active in using the amino acid mixture, protein, and NAG. In contrast, an average of only 7% of all SAR86 cells used amino acids and protein even in summer when DOM use was high. In addition to DOM uptake within a group, we explored the contribution of the three gammaproteobacterial groups to total community uptake of a compound. SAR86 contributed 5- to 10-fold less than the other gammaproteobacterial subgroups to the uptake of all compounds. We found that the overall contribution of the Ant4D3 clade to DOM uptake was highest, whereas the SAR86 clade contributed the least to DOM turnover in West Antarctic Peninsula waters. Our results suggest that the low growth activity of a bacterial clade leads to low abundance, fewer active cells and a low contribution to the turnover of DOM components.

A diverse community of heterotrophic bacteria processes dissolved organic material (DOM) in the Arctic Ocean and Antarctic seas, making up a critical part of the carbon cycle in polar environments. Some members of this community appear to be unique to polar waters, while others are also found in low-latitude oceans (1, 2). Single-cell methods have indicated that these bacteria are active in leucine incorporation (3), even though bulk estimates indicate that DOM fluxes mediated by heterotrophic bacteria are lower in polar waters than in other oceans (4). With few exceptions (5–7), however, previous single-cell studies and the bulk activity studies of polar systems have focused on leucine as a general index of bacterial activity (3, 8–13). The paucity of data about the uptake of other compounds by specific bacterial taxa hinders efforts to understand mechanisms in bacterial biogeography and the contribution of these bacteria to carbon cycling.

Studies examining DOM uptake by gammaproteobacteria, which make up 5 to 25% of the total bacterial community in polar systems and many other oceanic waters (14, 15), provide some examples of differences in the uptake of leucine and other DOM components among bacterial clades. Gammaproteobacteria took up more leucine and an amino acid mixture than ATP or glucose in Mediterranean coastal water according to single-cell studies (16, 17). Similarly, in the Arctic Ocean, gammaproteobacteria preferred amino acids to other types of low-molecular-weight (LMW) DOM, such as ATP or glucose (5, 6). However, the uptake of protein, algal derivatives, extracellular polysaccharides, and chitin by gammaproteobacteria was higher than their affinity for LMW DOM in the Delaware Bay and Mediterranean Sea (18–20). In Southern Ocean waters, adding peptone and algal extracts increased growth activity and turnover of amino acids and glucose by gammaproteobacteria (21).

Within the gammaproteobacteria, several subgroups are emerging as important players in marine surface waters, each with potentially different responses to DOM availability. One such subgroup is SAR86, a clade widely distributed from polar to temperate waters (1, 22), whose activity is just beginning to be quantified. The data on 16S rRNA levels suggest that growth activity of SAR86 is lower than SAR11 in temperate coastal waters (23). However, SAR86 may be active in other marine systems, according to results from a single-cell approach for leucine incorporation (24) and from a metatranscriptomic approach (25). SAR86 activity may be promoted by light since it has the gene for proteorhodopsin, a light-driven proton pump (26). However, despite being a cosmopolitan clade, the life strategy and contribution of SAR86 to DOM turnover are still unknown.

In contrast to SAR86, another gammaproteobacterial clade, Ant4D3, seems to have a polar biogeography (27, 28). The Ant4D3 clade has a relatively high level of activity in both Arctic and Antarctic coastal waters, and it may take up amino acids more than protein in polar coastal waters (10, 11). A gammaproteobacterial clade closely related to Ant4D3, Arctic96B-16 (29), also appears to be abundant and active in leucine incorporation in the Arctic Ocean and West Antarctic Peninsula (WAP) waters (9, 12). Previous studies suggest the potential for high DOM turnover in polar regions by various gammaproteobacterial subgroups, but DOM uptake by cosmopolitan and polar clades is still understudied.

In this study, we measured the uptake of various ^{3}H-DOM components by three gammaproteobacterial clades in WAP coastal waters in summer and fall. For the past 2 decades, the WAP marine ecosystem has been experiencing rapid warming and reduction in total sea ice cover in winter (30), which may signifi-

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cantly increase bacterial activity and carbon fluxes (4). Our goal was to determine whether differences in leucine incorporation previously observed among the three different clades (12) translate to differences in their contribution to the use of various types of DOM. In particular, we wanted to see whether DOM uptake by SAR86 is as low as indicated by leucine incorporation data (12). A previous study examined DOM uptake by the Ant4D3 clade in WAP waters (11), but no study has examined DOM uptake by SAR86 or Arctic96B-16 in polar systems. We found a high contribution to amino acid and protein use by Ant4D3 and Arctic96B-16, while the use of all ^{3}H-labeled compounds by the SAR86 clade was distinctly lower.

MATERIALS AND METHODS

Sample collection. Seawater samples were collected at a depth of 1 m at two sites during two consecutive austral summers (January 2011 and 2012) and one fall (May 2011) near Palmer Station, Antarctica. The two sampling sites are located 200 m (site B) and 3.2 km offshore (site E) from Palmer Station (http://pal.lternet.edu/). Surface water was pumped into 20-liter carboys and transported back to the lab in insulated containers. Details about water temperature, total prokaryote abundance, and leucine incorporation are reported elsewhere (12).

Abundance of gammaproteobacterial subgroups measured by CARD-FISH. Catalyzed reporter deposition-fluorescence *in situ* hybridization (CARD-FISH) was carried out as previously described (31), with some modifications (10). Briefly, seawater was fixed with paraformaldehyde at a 2% final concentration overnight at 4°C and then filtered onto 0.22-μm-pore-size polycarbonate filter membranes. Filters were stored at −80°C until use. Filters were coated with 0.01% (wt/vol) low-density agarose (MetaPhor agarose; Lonza Group, Switzerland) and incubated for 60 min in lysozyme solution. Filters were then hybridized overnight with horseradish peroxidase-conjugated FISH probes. Probes for three gammaproteobacterial subgroups were used: Ant4D3 (5′-CAAGCCAGGGC GTCGCCT-3′) (11), Arctic96B-16-196 (5′-GTCTGTAAACAGATCCCC TCCT-3′) (9), and SAR86-1249 (5′-GGCTTAGCGTCCGTCTG-3′) (31), with formamide concentrations in hybridization buffer at 45, 55, and 45%, respectively. The EUB338 probe for all bacteria and the NON338 nonsense probe were also used (32). Filters were rinsed, and tyramide signal amplification (TSA kit; Perkin-Elmer) of cyanine 3 dye (Cy3) was performed. Filters were rinsed again and dried before mounting on slides with a mixture of 4:1 Citifluor (Ted Pella) to Vectashield (Vector Laboratories) plus DAPI (4′,6′-diamidino-2-phenylindole) at 0.5 mg liter^{-1}.

Single-cell activity of bacterial subgroups measured by microautoradiography. Seawater was incubated with ^{3}H-labeled compounds immediately after collection. Paraformaldehyde-killed incubations were used as negative controls. The compounds examined included glucose, *N*-acetylglucosamine (NAG), a mixture of 15 amino acids, protein, chitin, and leucine. The glucose (10 to 20 Ci/mmol; Perkin-Elmer) and amino acids (55 Ci/mmol; American Radiolabeled Chemicals, Inc.) were added at a 0.5 nM final concentration and incubated for 4 h. The added concentration of ^{3}H-labeled NAG (60 Ci/mmol; American Radiolabeled Chemicals, Inc.) was 2 nM, and the incubation time was 8 h, whereas for [^{3}H]leucine (100 to 150 Ci/mmol; Perkin-Elmer) the final concentration was 20 nM incubated for 4 h. ^{3}H-labeled protein, made from *Vibrio alginolyticus* (18), was added at a final concentration of 65 ng per ml of seawater and incubated for 8 h. ^{3}H-labeled chitin oligomers were prepared as previously described (33). They were added at a final activity of 7.2×10^{-3} μCi/ml and incubated for 8 h. After the incubation, subsamples were filtered through polycarbonate filters and rinsed twice with filtered seawater. The total uptake of all compounds was estimated by radioassaying whole filters. Turnover rate constants were calculated from dividing the assimilated radioactivity by the added radioactivity and incubation time. Uptake was explored here without information about *in situ* concentrations of these compounds, which would affect the assimilation of the radiolabeled compounds.

Microautoradiography combined with CARD-FISH (MAR-CARD-FISH) was performed as previously described (12). In brief, filters from incubations with amino acids and leucine were exposed to photographic emulsion for 48 and 18 h, respectively. Filters for assaying uptake of protein, NAG, and chitin oligomers were exposed to photographic emulsion for 8 days. Single-cell use of glucose was not examined by microautoradiography because bulk uptake was very low (see Results), and single-cell use of glucose in WAP waters was undetectable during a previous study (11). In the paraformaldehyde-killed control, the percentage of cells with silver grains was <1%, and the size of grains around cells was <0.40 μm^2.

Imaging and MAR-CARD-FISH quantification. Samples were examined with an Olympus Provis AX-70 epifluorescence microscope, with a model 6720 AMC contoured passive air isolation plate (Electron Microscopy Sciences) to eliminate potential vibrations. Photographic exposure times for fluorescence and bright-field (microautoradiography) images were the same for all samples (34). Nonspecific binding by the NON338 probe was always <3% of the total cells. Silver grain areas (SGAs) were calculated as previously described (10). We used the Microbe Counter program (18) to quantify two sets of MAR-CARD-FISH data: one is the percentage of active cells within a group (those cells that are MAR-positive of all FISH probe-positive cells), and the second is the percent contribution of a group to all active cells taking up a compound (i.e., the cells that are FISH probe positive of all MAR-positive cells).

Statistical analysis. All percentages from single-cell analyses of substrate use were arcsine transformed prior to statistical analyses. Since silver grain areas varied >10-fold, they were log transformed prior to analyses.

RESULTS

We repeatedly sampled two sites in WAP coastal waters during month-long trips in two summers and one fall. The environmental properties of the sampled waters are summarized elsewhere (12). Because the number of total prokaryotes, bacterial production, and environmental parameters such as temperature and nutrient concentrations did not differ substantially between the two sites, the results were analyzed together.

Uptake of LMW DOM. Uptake of four LMW compounds by the bacterial community in WAP waters varied depending on the compound, season, and year. Average turnover rate constants from highest to lowest were as follows: amino acids, leucine, glucose, and NAG (Table 1). Because leucine was added at a higher final concentration (20 nM) than the amino acid mixture (0.5 nM), its turnover rate constant was on average 10-fold lower than the rate constant of the amino acid mixture during all three sampling trips. The rate constant for glucose turnover was lower than that for the amino acid mixture by 1.8-fold in the summer of 2011 and 3-fold in the summer of 2012. The rate constant for NAG was the lowest of the four LMW compounds, with turnover rate constants 5- to 10-fold lower than those for glucose, the compound with the next to slowest turnover. Turnover rate constants in summer were significantly higher than in fall for leucine ($P < 0.0001$, Student *t* test), the amino acid mixture ($P < 0.01$), glucose ($P < 0.0001$), and NAG ($P < 0.05$). The leucine turnover rate constant was slightly lower in summer 2011 than in summer 2012 ($P < 0.01$), whereas in contrast the turnover of glucose was almost 5-fold higher in summer 2011 than in summer 2012 ($P < 0.0001$). These data indicate that the compounds we examined were used more in the summer than the fall with substantial interannual variation and that they were not used equally.

TABLE 1 Average turnover rate constants for bulk uptake of [^{3}H]leucine, amino acid mix, *N*-acetylglucosamine, and glucose in summer (January) and fall (May)[a]

	Avg turnover rate constant (10^{-3} h^{-1}), SE, and no. of samples											
	Leu			AA			NAG			Gluc		
Expedition	Avg	SE	*n*	Avg	SE	*n*	Avg	SE	*n*	Avg	SE	*n*
Summer 2011	1.1	0.14	14	30.8	4.7	14	ND	ND	0	16.7	2.32	14
Fall 2011	0.2	0.03	12	7.6	1.0	12	0.24	0.07	8	2.6	0.39	8
Summer 2012	1.9	0.18	14	10.7	2.7	14	0.74	0.27	6	3.6	0.76	6

[a] Average turnover rate constants for the bulk uptake of [^{3}H]leucine (Leu), amino acid mix (AA), *N*-acetylglucosamine (NAG), and glucose (Gluc) in summer (January) and fall (May) are presented. The number of samples (*n*) includes trips to two sites on different days during the indicated season. ND, not done.

Single-cell uptake of DOM by gammaproteobacterial clades. We compared leucine assimilation to uptake of the amino acid mixture by the three clades (Table 2). Cells within the Ant4D3 and Arctic96B-16 clades were equally active for the uptake of leucine and of the amino acid mixture in both summer and fall (67% ± 7% of cells within a group). Use of both compounds by SAR86 was 1.5-fold to 10-fold lower than use by the other two clades of gammaproteobacteria. Although the use of the amino acid mixture by SAR86 was equal to its use of leucine in fall (18.8% ± 6%), leucine use by this clade was 3-fold greater than amino acid use in summer. In general, however, single-cell use of amino acids was comparable to single-cell use of leucine by a clade.

In order to evaluate the single-cell use of LMW DOM versus high-molecular-weight DOM, we compared the uptake of monomeric substrates to uptake of their polymeric counterparts. For the three clades, single-cell use of amino acids was generally higher than protein use within the clades. In summer, 60 to 75% of the cells within the Ant4D3 and Arctic96B-16 clades used amino acids, while only 15 to 20% used protein (Fig. 1A). However, the use of amino acids did not differ from protein use by the SAR86 clade in summer, and on average 7% ± 3% of SAR86 cells were active in taking up the amino acid mixture or protein. Similar to summer, use of the amino acid mixture by Ant4D3 and by all bacteria in fall was 2- to 10-fold higher than protein use (Fig. 1B). Use of amino acids by Arctic96B-16 in fall ranged widely (7 to 65%) and did not differ significantly from protein use ($P > 0.05$, Student *t* test). Use of amino acids was higher than protein use by SAR86 as no cells detected within this clade took up protein in fall. Amino acid and protein use by this clade did not change between summer and fall.

Chitin use was low and nearly undetectable; often only one to five cells in an analysis of a clade were positive for ^{3}H-labeled chitin uptake (data not shown). Consequently, MAR-CARD-FISH of this compound was not examined further.

Overall, the single-cell use of NAG was lower than use of the amino acid mixture. The percentage of cells using NAG was only 8 to 12% in summer and 1 to 10% in fall for all bacterial groups (Fig. 2), which was much lower than the percentage of cells using the amino acid mixture in both seasons (10 to 75%) (Fig. 1). The uptake of NAG was 3- to 5-fold higher in summer than in fall by the Ant4D3 clade ($P < 0.01$) and general bacteria ($P < 0.05$), while there was no difference between the two seasons for Arctic96B-16 (Fig. 2). The fraction of SAR86 cells taking up NAG was only ca. 3% in summer, much lower than that for the other two groups. However, in fall the fraction of cells using NAG was equally low (1 to 3%) for all three bacterial groups.

Single-cell use of DOM measured by silver grain area. Uptake of ^{3}H-labeled DOM by individual cells can be explored using silver grain area (SGA) around an active cell. The SGA for all bacteria

TABLE 2 Percent active within groups for the uptake of leucine and the amino acid mix[a]

	Avg % active of probe-labeled cells (SE) within groups							
	Summer				Fall			
	Leucine		Amino acids		Leucine		Amino acids	
Clade	Avg	SE	Avg	SE	Avg	SE	Avg	SE
Ant4D3	61.3	6.7	63.2	2.6	63.5	8.9	62.0	4.3
Arctic96B-16	75.4	7.6	79.1	3.1	69.4	9.4	53.5	4.6
SAR86	24.6	7.3	6.9	3.5	26.7	6.5	10.8	4.3

[a] For leucine, the number of samples was 12 in summer and 6 in fall. For amino acids, the number of samples was 8 for both summer and fall.

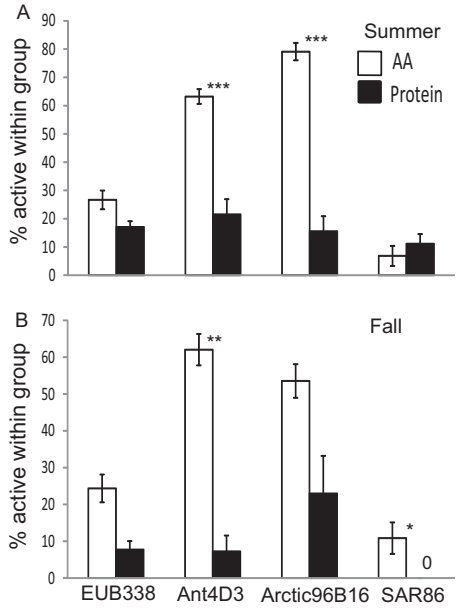

FIG 1 Percent activity for all bacteria (EUB338) and three gammaproteobacterial clades for the use of amino acids and protein in summer (A) and fall (B). Eight samples were used to examine amino acid use in both seasons. Five and three samples were used to examine protein use in summer and fall, respectively. Error bars indicate the standard errors. No uptake of protein by SAR86 was detected in fall. Asterisks denote significant difference between amino acid and protein. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

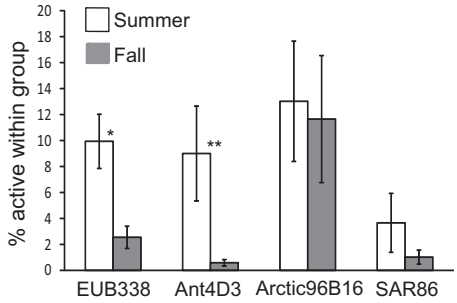

FIG 2 Percent activity for all bacteria (EUB338) and three gammaproteobacterial clades for the use of NAG in summer (white bars) and fall (gray bars). Bars are based on four samples in summer and four in fall. Error bars indicate the standard errors. Asterisks denote significant difference between summer and fall. **, $P < 0.01$; *, $P < 0.05$.

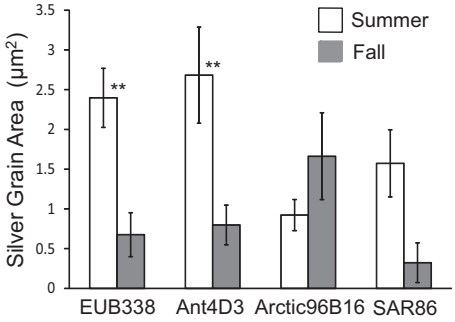

FIG 4 Average SGA of cells active for the use of NAG in summer (white bars) and fall (gray bars). Bars are based on all silver grain- and probe-positive cells. Error bars indicate the standard errors. **, $P < 0.01$.

(Eub338 probe) was significantly larger for amino acid uptake than the protein uptake SGA in summer (Fig. 3A), similar to the pattern in the percentage data (Fig. 1A). In contrast, the average SGA for protein use by active Ant4D3 and Arctic96B-16 cells was larger than that for amino acids in summer ($P < 0.00001$) (Fig. 3A). In summer, the SGA for SAR86 cells using amino acids and protein was not significantly different ($0.68 \pm 0.2\ \mu m^3$). The SGA for single-cell use of amino acids by SAR86 was 2- to 3-fold smaller than that for Ant4D3 and Arctic96B-16 ($P < 0.001$). The difference between the percent active data (Fig. 1A) and the SGA data (Fig. 3A) suggest that although a greater number of Ant4D3 and Arctic96B-16 cells used amino acids than protein in summer, cells that were active for protein uptake incorporated more protein on average than cells taking up amino acid.

In fall, the pattern in SGA for amino acid and protein uptake was reversed. In contrast to summer, the SGA for amino acid use was significantly larger in fall by 3-fold than the SGA for protein

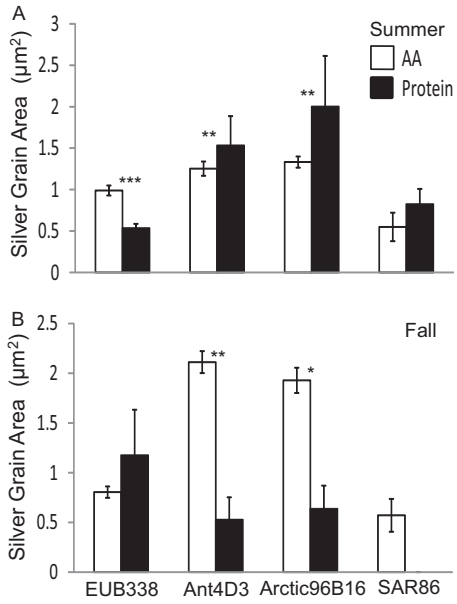

FIG 3 Average SGA of cells active for amino acids and protein in summer (A) and fall (B). Error bars indicate the standard errors. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

use for cells in the Ant4D3 and Arctic96B-16 clades (Fig. 3B). The amino acid use reflected in SGA for both clades was larger than that for all bacterial cells. No cells in the SAR86 clade used protein in the fall. Interestingly, the SGA for active cells in the two gammaproteobacterial clades using protein was 3-fold smaller in fall than summer.

Average SGA of cells active for NAG uptake was generally larger in summer than fall, except for the Arctic96B-16 clade (Fig. 4). SGA of Ant4D3 and Arctic96B-16 cells was 2.5-fold larger in summer than for cells using NAG in the SAR86 clade. Average SGA for SAR86 cells taking up NAG in summer and fall ranged widely between $1.57 \pm 0.2\ \mu m^3$ and $0.32 \pm 0.4\ \mu m^3$ and was not significantly different than the SGA for the other groups.

Contribution by gammaproteobacterial clades to DOM uptake. Single-cell MAR-CARD-FISH data can be analyzed to determine the contribution of clades to total uptake of compounds, in addition to exploring the relative number of cells within a group taking up a compound. The percent contribution of all three clades to the total community uptake of amino acids was generally higher than their percent abundance suggested (Fig. 5A). For example, the Ant4D3 clade contributed more to uptake of amino acids in summer (42%) and fall (10%) than their relative abundance (18 and 4% in summer and fall) within the community. The contribution of this clade to total amino acid uptake in summer was 4-fold higher than in fall ($P < 0.01$). In general, percent contribution of Arctic96B-16 (36%) to total amino acid uptake was equivalent to the contribution by the Ant4D3 clade in summer, both being greater than suggested by their percent abundance within the community (Fig. 5A). In contrast, contribution by SAR86 to bulk amino acid uptake was low (2%) in both summer and fall and always below its abundance in the community (Fig. 5A).

The contribution by Ant4D3 to total protein use was high (27%), almost equal to its contribution to total amino acid use in summer (Fig. 5B). The Ant4D3 clade contributed to protein uptake 2-fold more than did the Arctic96B-16 clade (11%) and 16-fold more than did the SAR86 clade (1.6%) in summer. However, in fall, the Ant4D3 and Arctic96B-16 clades accounted for equally low fractions of protein use (Fig. 5B).

The contribution to NAG use by Ant4D3 and Arctic96B-16 clades was similar, both averaging ca. 40% in summer and 2% in fall (Fig. 6). Consistent with the relatively low number of cells in the SAR86 clade active in using the other compounds, the percent contribution of SAR86 to NAG uptake was ~4-fold less than the

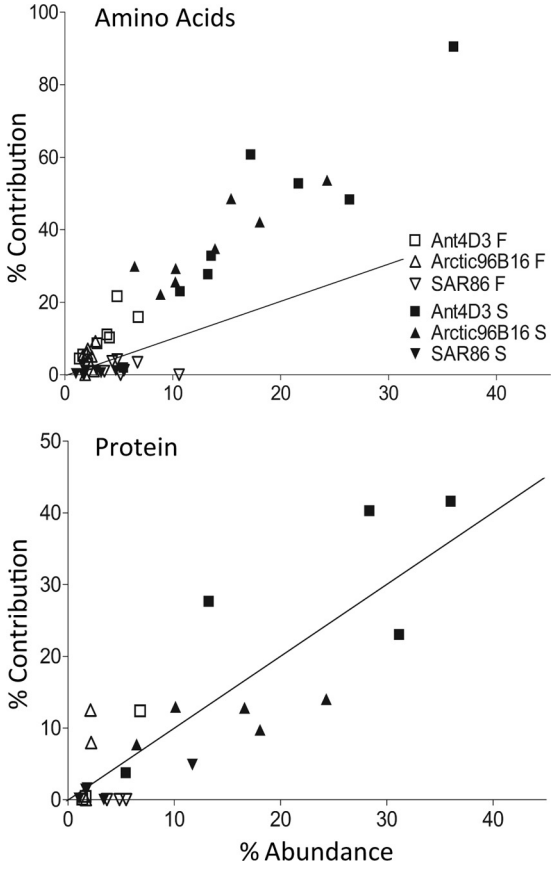

FIG 5 Percent contribution of the groups to amino acid and protein uptake versus the relative abundance of the groups (i.e., the percentage of total prokaryotic abundance). Each symbol is a single sample. Open symbols indicate fall values (F), and filled symbols indicate summer (S) values. The 1:1 lines are shown.

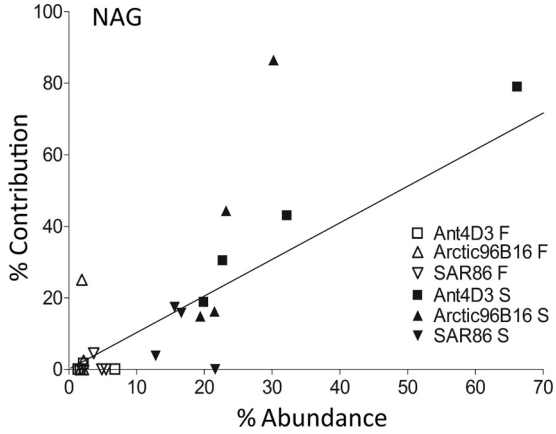

FIG 6 Percent contribution of the groups to NAG use versus the relative abundance of the groups (i.e., the percentage of total prokaryotic abundance). Each symbol is a single sample. Open symbols are fall (F) values, and filled symbols are summer (S) values. The 1:1 line is shown.

contribution by other groups. However, in summer, the SAR86 contribution to total NAG use was almost 15-fold higher than its contribution to amino acid use and 5-fold higher than its contribution to protein use.

DISCUSSION

Our goal was to evaluate the uptake of DOM by slow and fast-growing gammaproteobacterial clades in the WAP coastal waters. We measured turnover rates and used MAR-CARD-FISH to explore single-cell uptake of an amino acid mixture, glucose, NAG, and protein by the Ant4D3, Arctic96B-16, and SAR86 clades. Both Ant4D3 and Arctic96B-16 clades, previously found to be highly active in leucine assimilation (12), also took up amino acids, protein, and NAG at high levels. In contrast, the SAR86 clade which was not active in leucine assimilation (12) incorporated less DOM of all types in both seasons. The overall contribution to DOM turnover differed significantly among clades.

The four LMW compounds examined in the present study were chosen for single-cell analyses because previous reports had found wide variation in total uptake, similar to our results. For example, other studies also found high assimilation of amino acid mixtures (5, 35, 36). Amino acids may be preferred over other LMW compounds because they fulfill requirements for both nitrogen and carbon (36). In fact, dissolved free amino acids can support over 50% of bacterial production in marine waters (36). The turnover of NAG, another nitrogen-containing compound, was slow in WAP waters (turnover time of 236 days) but faster (6 days) in Californian coastal waters (37). Amino acid use was higher than glucose use in WAP waters, a finding consistent with previous estimates of low glucose uptake in these waters (11). Bulk turnover rates varied between the 2 years we examined, as did chlorophyll and other biogeochemical properties (data not shown). This interannual variation is well known for this polar system and is ultimately tied to the Southern Oscillation and climate change forcing (38).

We compared DOM uptake, in particular the use of an amino acid mixture, to leucine incorporation because it is often used as a general index of growth and protein synthesis. In WAP waters, Ant4D3 and Arctic96B-16 cells were equally active in using the amino acid mixture and in incorporating leucine. Similar results for Ant4D3 were found by a previous study of WAP waters and in the Beaufort Sea in summer (10, 11). In contrast, uptake of the amino acid mixture by SAR86 cells was low, perhaps simply because it was not very active, as suggested by the leucine data (12). Another factor may be the lower affinity of SAR86 bacteria for amino acids (39). Genomic data suggest that these bacteria may have a reduced ability to assimilate amino acids due to lack of ABC-type transporter genes, but they may have a higher affinity for higher-molecular-weight compounds (39).

The high uptake of amino acids and protein by the Ant4D3 and Arctic96B-16 clades may indicate that these two groups prefer exogenous nitrogen compounds. This hypothesis is supported by sequence data from fosmid clones indicating that the Ant4D3 group has fewer pathways for the synthesis of common amino acids (29). Although more cells in the Ant4D3 and Arctic96B-16 clades used amino acids than protein in both summer and fall, single-cell activity measured by SGA indicated that active Ant4D3 clade cells used protein more than amino acids in summer; SGA was larger for cells actively taking up protein. Similar results were reported previously (11). The active cells in this clade and in Arctic96B-16 may prefer to take up protein more so than amino acids. Consistent with our results, gammaproteobacteria take up protein and algal extracts more than other types of DOM in waters

of the North Atlantic Ocean and Mediterranean Sea (18, 19). Our results suggest a possible switch to protein use in summer when the input of dissolved protein is high coupled to the high phytoplankton biomass typical of these waters in summer (35, 40, 41). We expected similar results for Ant4D3 and Arctic96B-16 clades because their growth-related activity was similar in WAP waters (12) and because they are closely related (29). DOM uptake by these two closely related clades differed occasionally but usually was similar. Genomic information for these two clades would be useful for exploring their capacity for using different DOM compounds.

Single-cell use of NAG was significantly higher than chitin uptake by gammaproteobacteria in WAP waters (the present study), unlike gammaproteobacteria in Delaware Bay waters which preferred chitin to NAG (18). Chitin uptake was almost undetectable using single-cell methods in WAP waters, potentially due to several reasons. Chitin is often bonded with other biomolecules and must be broken down and hydrolyzed before uptake, a process which may result in loss of these monomers to the extracellular environment before uptake can occur (42). However, chitin hydrolysis and uptake by gammaproteobacteria in the Delaware Bay were detectable by the same methods as used here (33, 43), and we expect mechanisms of chitin uptake to be similar in the WAP and other marine waters. Other evidence indicates that chitinolytic bacteria may be rare in WAP waters and that these bacteria may be more prominent in Antarctic marine sediments (44). Surprisingly, the SGA of NAG-active SAR86 cells was larger than the SGA for amino acid use in summer, a result that is consistent with the number of genes for transporters in the SAR86 genome (39). The SAR86 genome includes a relatively high number of TonB-dependent outer membrane receptors, which catalyze transport of compounds such as NAG (39).

However, overall SAR86 was not very active in taking up the compounds we examined, and the contribution by this clade to DOM use was always lower than suggested by their percent abundance within the community. In contrast, we found a much higher percent contribution by the Ant4D3 and Arctic96B-16 clades to DOM uptake than their percent abundance might suggest, and these two groups likely play a much larger role in DOM flux than SAR86 in the WAP coastal ecosystem.

The low DOM uptake by SAR86 measured in the present study is consistent with the low leucine incorporation levels reported previously (12), indicating that these bacteria do not grow rapidly relative to the two other gammaproteobacterial clades and to the total bacterial community in WAP waters. Another study using 16S rRNA levels also found low relative activity of SAR86 in Mid-Atlantic Bight coastal waters (23). These data suggest that the SAR86 clade is a defensive strategist, maintaining low growth rates to defend against grazers and viruses. The result could be its widespread distribution in spite of low activity. In contrast, leucine incorporation by SAR86 was high in the North Sea (24), and the clade was well represented in metatranscriptomes in southeast U.S. coastal waters (25). SAR86 may be active in these waters because they are exposed to compounds in the labile DOM that are optimal for its growth. Along with the differences in SAR86 activity among these regions, the phylogenetic composition and metabolic potential of the SAR86 clade also varies (45). More data are needed to determine whether the SAR86 clade is in fact a defensive strategist or if diversity within the clade prevents any generalization to be made.

This study adds to the growing data indicating that bacterial clades differ in uptake of different DOM components and that this difference is tied to growth strategies. Although more widespread in the world's oceans than the Ant4D3 and Arctic96B-16 clades, the SAR86 clade is less abundant, appears to grow more slowly, and appears to contribute less to DOM uptake than the Ant4D3 and Arctic96B-16 clades in WAP waters. Overall, our results highlight the need to connect specific bacterial clades to the uptake of specific DOM components in order to better understand the biogeography, ecological niches, and roles of bacteria in the carbon cycle.

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