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Utilization of Organic Nitrogen Sources by Two Phytoplankton Species and a Bacterial Isolate in Pure and Mixed Cultures

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Algal production of dissolved organic carbon and the regeneration of nutrients from dissolved organic carbon by bacteria are important aspects of nutrient cycling in the sea, especially when inorganic nitrogen is limiting. Dissolved free amino acids are a major carbon source for bacteria and can be used by phytoplankton as a nitrogen source. We examined the interactions between the phytoplankton species *Emiliania huxleyi* and *Thalassiosira pseudonana* and a bacterial isolate from the North Sea. The organisms were cultured with eight different amino acids and a protein as the only nitrogen sources, in pure and mixed cultures. Of the two algae, only *E. huxleyi* was able to grow on amino acids. The bacterium MD1 used all substrates supplied, except serine. During growth of MD1 in pure culture, ammonium accumulated in the medium. Contrary to the expectation, the percentage of ammonium regenerated from the amino acids taken up showed no correlation with the substrate C/N ratio. In mixed culture, the algae grew well in those cultures in which the bacteria grew well. The bacterial yields (cell number) were also higher in mixed culture than in pure culture. In the cultures of MD1 and *T. pseudonana*, the increase in bacterial yield (number of cells) over that of the pure culture was comparable to the bacterial yield in mixed culture on a mineral medium. This result suggests that *T. pseudonana* excreted a more-or-less-constant amount of carbon. The bacterial yields in mixed cultures with *E. huxleyi* showed a smaller and less consistent difference than those of the pure cultures of MD1. It is possible that the ability of *E. huxleyi* to use amino acids influenced the bacterial yield. The results suggest that interactions between algae and bacteria influence the regeneration of nitrogen from organic carbon and that this influence differs from one species to another.

Phytoplankton and heterotrophic bacteria are major trophic levels in marine pelagic food webs. Phytoplankton are the primary producers of organic material, and bacteria are the main consumers. There is a direct interaction between the two groups of organisms: bacteria mineralize nutrients (9) and make them available for algal use.

Nitrogen is considered to be the most important macronutrient in the control of growth of marine phytoplankton (14, 26). In spring, most of the nitrogen in seawater is present as nitrate. During the growth season, autotrophic and heterotrophic organisms use nitrate for growth and convert it into other forms of nitrogen, notably organic nitrogen and ammonium. In summer, nitrate is depleted in large parts of the surface waters, concentrations of dissolved organic nitrogen compounds often being higher than the concentration of inorganic nitrogen (5). Under such conditions, it would be advantageous for phytoplankton to have the ability to use organic nitrogen sources in addition to inorganic nitrogen. A large portion of the organic nitrogen consists of dissolved free amino acids (DFAA) and dissolved combined amino acids (DCAA). Many species of marine algae have been shown to use amino acids and other organic nitrogen sources in laboratory culture (for a review, see reference 2). However, the quantitative importance of this uptake in the field is still subject to debate (8, 11, 30).

There is increasing evidence that amino acids supply a large fraction of the carbon and energy needs for bacterial growth in the marine environment (13, 20–22, 36). The support of bacterial growth by DCAA is less well studied, but turnover times of protein are comparable to those for DFAA (19). This is in agreement with uptake studies by Coffin (6) and Wheeler and Kirchman (38) that indicate that DCAA and DFAA are roughly of equal importance to bacteria as sources of carbon and energy. DCAA can be converted into DFAA through hydrolysis by proteases. Depending on the type of proteolytic system, amino acids will be released into the surrounding medium (32). Hollibaugh and Azam (19) come to the conclusion that net liberation of DFAA from DCAA in oceanic waters is not significant, indicating a close coupling between the hydrolysis of DCAA and the uptake of DFAA.

Part of the nitrogen in amino acids taken up by bacteria is mineralized and excreted in the form of ammonium. The fraction remineralized is a function of the C/N ratio of the nitrogenous substrate and of the bacteria as well as a function of the bacterial growth yield on the substrate (15, 16).

Both remineralized ammonium and amino acids are potential nitrogen sources for phytoplankton growth, and their fluxes are directly influenced by the interactions between phytoplankton and bacteria. In this study we investigated the influence of alga-bacterium interactions on nitrogen fluxes by culturing phytoplankton and bacteria in pure and mixed cultures. We examined the role of amino acids as phytoplankton N sources, the fraction of nitrogen liberated by bacteria from DFAA and protein, and the influence of interactions between algae and bacteria on growth and regeneration rates. We chose to work with monocultures of bacterium-free strains of the abundant prymnesiophyte *Emiliania huxleyi* and the diatom *Thalassiosira pseudonana* without or with a strain of a marine heterotrophic bacterium isolated on an algal culture filtrate of the diatom *Thalassiosira excentrica*.

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MATERIALS AND METHODS

A monoculture of the alga *E. huxleyi* L was obtained from P. Westbroek of the University of Leiden, Leiden, The Netherlands. This calcifying strain was originally isolated from Oslo Fjord by E. Paasche. The algae were cultured in the medium of Admiraal and Werner (1), which was used in all experiments described in this study. Upon inspection with epifluorescence microscopy, the culture was found to contain bacteria. The culture was made axenic by washing the algal cells by gentle centrifugation and resuspension in sterile medium, followed by incubation with the antibiotics carbenicillin and cefotaxime (500 μg/ml; Sigma, St. Louis, Mo.). Every 24 h, serial dilutions were made into fresh medium without antibiotics. After growth of the algae was observed, the cultures were checked for the presence of bacteria by epifluorescence microscopy and by inoculation (50% inoculum) into sterile medium containing 0.05% (wt/vol) peptone (BBL) and 0.03% (wt/vol) yeast extract (BBL). In this way, an axenic culture of strain L was finally obtained from a dilution of the culture incubated for 48 h in the antibiotic-containing medium. An axenic culture of *T. pseudonana* 3H was obtained from the Culture Collection of Marine Phytoplankton in West Boothbay Harbor, Maine.

The bacterial strain, an unidentified nonmotile rod referred to as MD1, was isolated from the North Sea off the Dutch coast. This water sample was diluted serially and plated on agar plates prepared by solidifying an algal culture filtrate with 1.5% agar. The filtrate was obtained by filtering a late-log-phase culture of *T. excentrica* through a 0.2-μm-pore-size cellulose acetate filter (Sartorius, Göttingen, Federal Republic of Germany). Care was taken not to apply strong suction to avoid cell breakage. Algal extracellular dissolved products were thus used as the substrate in an attempt to favor the isolation of ecologically relevant bacterial species. MD1 was isolated from the highest dilution showing growth (i.e., 10^5).

Good growth of the two species of algae was obtained in the culture medium, and stock cultures were maintained by transferring 5 ml of a growing culture into 75 ml of fresh medium every 8 weeks. Algal cultures were tested for the presence of bacteria by epifluorescence microscopy and enrichment culture before each transfer of stock cultures, as well as at the beginning and end of each experiment. Cultures remained axenic and grew well over a period of more than 2 years. No change in the growth rate was seen after the nonaxenic culture of *E. huxleyi* was made free of bacteria when cultured in a mineral medium.

During the experiments, the algae and bacteria were cultured in the artificial seawater medium of Admiraal and Werner (1), containing 36 μM phosphate and 50 μM nitrogen, either as ammonium chloride or as an amino acid or protein. The substrates used were the amino acids alanine, aspartate, asparagine, glutamate, glutamine, glycine, leucine, and serine, and the protein bovine serum albumin (BSA). The two algal strains and bacterium MD1 were grown separately and in mixed culture on all these substrates. Incubations were performed in 100-ml glass serum bottles at 15°C with an illumination of approximately 100 microeinsteins m^{-2} s^{-1} (14 h of light and 10 h of dark) in a culture cabinet. Cells were kept in suspension by slowly revolving the culture bottles on a rolling device (±3 rpm). All glassware used had been baked overnight at 500°C to minimize contamination by organic substances other than the substrates added.

Samples for cell counts were fixed in glutaraldehyde (1% [vol/vol] final concentration). Samples for the determination of ammonium and amino acids were centrifuged (14,000 × *g*, 10 min), and the supernatant was stored frozen at −20°C until analysis.

Analyses. Amino acids were determined by high-performance liquid chromatography (HPLC). The HPLC method used is a modification of the method described by Lindroth and Mopper (25). Ten microliters of the internal standard α-aminobutyric acid was added to 500 μl of sample in an Eppendorf plastic reaction cup, yielding an appropriate standard concentration (200 nM to 10 μM), depending on the expected concentrations in the sample. Twenty microliters was placed in a 100-μl glass insert in an autosampler vial (Alltech, Deerfield, Ill.). The vials were placed in an LKB Pharmacia 2151-010 autosampler. Derivatization was performed automatically by the autosampler. The derivatization was started by adding 5 μl of reagent, made freshly every day by mixing 500 μl of borate buffer (0.4 M, pH 10) with 50 μl of *o*-phthalaldehyde solution (50 mg in 1 ml of methanol) and 5 μl of 2-mercaptoethanol. After 90 s, 5 μl of 5% acetic acid was added to terminate the derivatization reaction. Twenty microliters was injected in a Kratos gradient HPLC system equipped with a Jasco 821-FP fluorescence detector. The amino acid derivatives were separated on a 15-cm-long Alltech Adsorbosphere OPA-HR column with a linear gradient from 10 to 52% solvent B in 20 min. Solvent A was a sodium acetate buffer adjusted to pH 5.9 with acetic acid. To this buffer, 2% (vol/vol) tetrahydrofuran was added. Solvent B was a mixture of 10% (vol/vol) acetonitrile in methanol. All solvents were HPLC grade (Rathburn). Milli-Q ultrapure water was used throughout the whole procedure, and if available, chemicals specially purified for amino acid analysis (BDH Sepramar, Poole, United Kingdom) were used.

Ammonium was measured on an autoanalyzer by standard techniques (17). In all cultures, microbial cells were counted by epifluorescence microscopy (18). The bacteria were stained with Hoechst 33258 (Sigma) at a final concentration of 50 μM.

RESULTS

Figure 1 shows the final cell counts after 4 weeks of growth of the axenic cultures of *E. huxleyi* and *T. pseudonana* in the standard growth medium supplemented with 50 μM nitrogen in the form of various amino acids, BSA, or ammonium chloride. Both species of algae grew well with ammonium chloride as the sole source of nitrogen. In addition, *E. huxleyi* showed considerable growth with alanine or leucine as the N source but only slight growth when N was supplied in the form of serine, glutamine, glycine, asparagine, or BSA. *T. pseudonana* did not grow significantly with nitrogen supplied in the form of any of the amino acids tested or BSA as the N source. Very weak growth occurred when alanine was supplied in the medium.

Bacterial strain MD1 was a gram-negative isolate from high dilutions (10^5) of North Sea water. This bacterium is a nonmotile rod 0.8 to 3 μm long. The GC content is 63.7%. The strain is catalase and oxidase positive. Growth of MD1 was tested on a number of substrates. The strain showed growth with the carbohydrates fructose, glucose, xylose, and starch, the amino acids alanine, aspartate, asparagine, glutamate, glutamine, glycine, leucine, lysine, and phenylalanine, the proteins casein and BSA, and the fatty acids acetate, malonate, and α-ketoglutonate. No growth was detected with serine, threonine, glycollate, and DNA.

In Table 1, the cell densities and concentrations of substrate and ammonium are given for the pure cultures of MD1 and the mixed cultures. These data were used to calculate the yields (number of cells) per micromole of amino acid nitrogen and

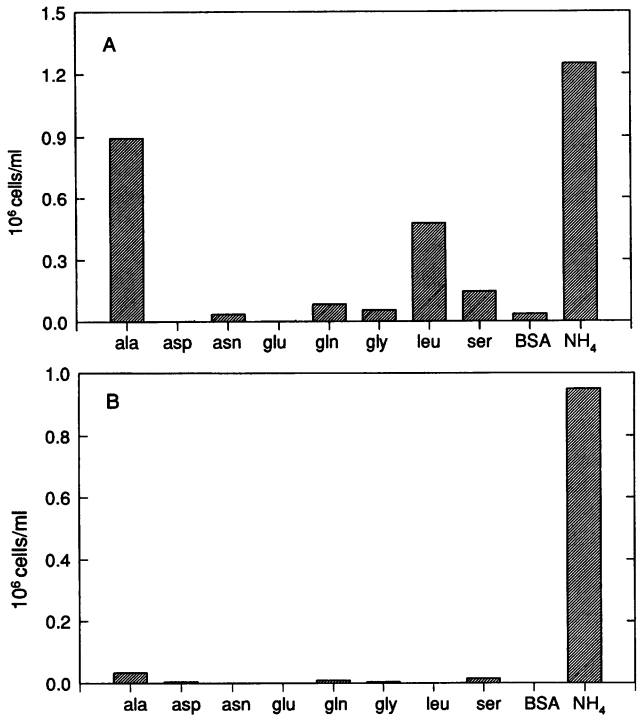

FIG. 1. Cell densities of *E. huxleyi* (A) and *T. pseudonana* (B) after 4 weeks of growth on amino acids, BSA, and NH_4.

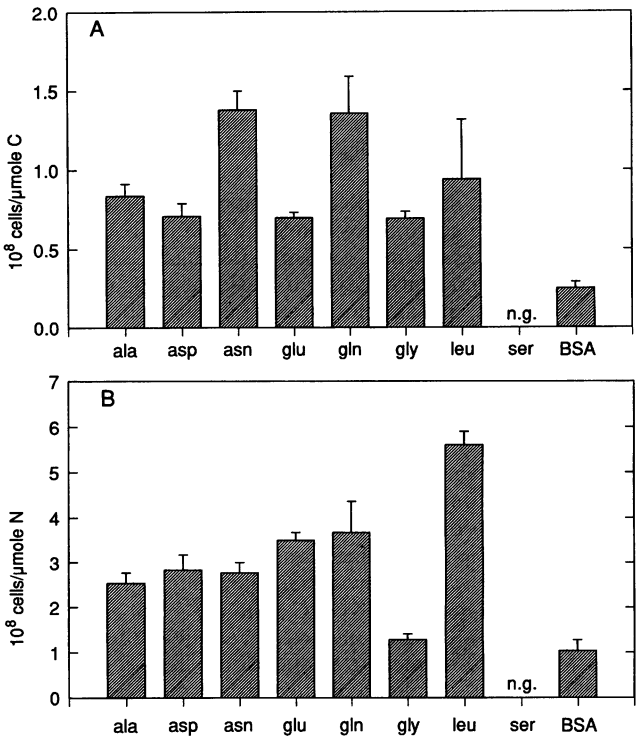

FIG. 2. Yields (cell number) of MD1 per micromole of amino acid carbon taken up (A) and per micromole of amino acid nitrogen taken up (B) in pure culture. Bar heights represent means and error bars represent standard deviations of two separate experiments. n.g., no growth.

amino acid carbon taken up from the culture medium. The difference between the initial and final substrate concentrations was the amount of substrate taken up. The normalized cell yields are shown in Fig. 2 to 5.

Bacterial strain MD1 used all organic N sources tested as sources of carbon, nitrogen, and energy, except for serine (Fig. 2). As expected, MD1 showed no growth in the medium containing ammonium chloride as the N source without a carbon and energy source (negative control). After growth of MD1 in the pure culture with the organic nitrogen sources had stopped, cell numbers of MD1 were counted and the concentrations of ammonium and free amino acids in the spent culture liquid were determined. The average yield (cell number) of MD1 in the successful cultures (Fig. 2) with amino acids as the substrates was 0.825×10^8 cells per μmol of carbon taken up (supplied as amino acid C), with asparagine giving the highest value (1.5×10^8) and BSA giving the lowest value (0.3×10^8). Bacterial yields per micromole of amino acid nitrogen

TABLE 1. Cell densities and substrate and ammonium concentrations at the end of the batch culture experiments with pure and mixed cultures[a]

Substrate	MD1			MD1 + *E. huxleyi*				MD1 + *T. pseudonana*			
	Cell density (10^6 cells ml^{-1})	Amino acid concn (μM)	NH_4^+ concn (μM)[b]	Cell density (10^6 cells ml^{-1})		Amino acid concn (μM)	NH_4^+ concn (μM)	Cell density (10^6 cells ml^{-1})		Amino acid concn (μM)	NH_4^+ concn (μM)
				Algae	Bacteria			Algae	Bacteria		
Ala	12.7 (1.2)	0	29.3	0.56 (0.05)	17.2 (0.6)	0	0.9 (0.3)	0.62 (0.05)	18.2 (3.4)	0	0.5 (0.5)
Asp	14.2 (1.7)	0	10.5	0.83 (0.05)	20.9 (14.0)	0	1.3 (0.5)	0.86 (0.1)	44.3 (4.4)	0	0.6 (0.6)
Asn	13.9 (1.2)	0	9.6	0.59 (0.06)	13.4 (2.0)	0	2.0 (0.1)	0.61 (0.43)	22.3 (2.4)	0.7 (0.7)	0.9 (0.1)
Glu	17.5 (0.9)	0	16.0	0.20 (0.12)	20.8 (6.7)	0	1.5 (0.3)	0.51 (0.08)	25.7 (2.3)	0	1.4 (0.5)
Gln	18.4 (3.4)	0	37.3	1.07 (0.39)	18.6 (8.0)	0	0.6 (0.4)	0.35 (0.27)	22.4 (9.6)	0	1.9 (0.2)
Gly	6.5 (0.6)	1.4 (0.2)	26.8	0.67 (0.10)	11.1 (6.1)	0	0.3 (0.3)	0.64 (0.02)	17.9 (3.1)	0	1.4 (0.5)
Leu	28.0 (1.5)	2.0 (0.1)	18.9	0.24 (0.10)	29.5 (13.1)	0	1.7 (0.9)	0.31 (0.03)	37.1 (9.0)	0	2.8 (0.2)
Ser	NG[c]	50	0	0.5 (0.31)	3.1 (2.9)	28.4 (3.3)	3.8 (1.2)	0.12 (0.02)	7.3 (5.9)	36.6 (0.2)	1.6 (0.1)
BSA	5.2 (1.2)	0	6.7	0.19 (0.08)	8.1 (5.6)	0	0.8 (0.8)	0.08 (0.02)	15.5 (1.0)	0	2.0 (0.5)
NH_4^+	NG	0	50	1.11 (0.01)	8.8 (3.1)	0	0.6 (0.4)	0.64 (0.28)	10.2 (0.9)	0	1.1 (0.1)

[a] At the beginning of the experiments, 50 μM nitrogen was present, either as ammonium or as organic nitrogen. Each value is the mean of two experiments, with the standard deviation given in parentheses. If the concentrations were zero in both experiments, no standard deviation is given.

[b] One series of ammonium samples was lost, so no standard deviations could be calculated for ammonium in the pure MD1 cultures.

[c] NG, no growth.

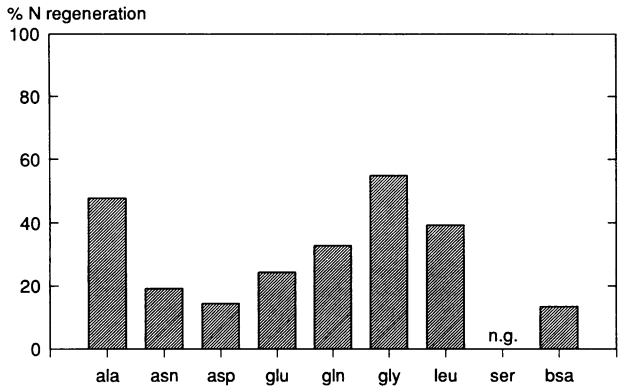

FIG. 3. Fraction of amino acid nitrogen taken up by MD1 in pure culture regenerated as ammonium at the end of the growth phase. n.g., no growth.

taken up varied somewhat more, with glycine giving the lowest value (1.5×10^8) and leucine giving the highest value (5×10^8).

After growth of MD1 on BSA or on the single amino acids that supported growth had ceased, there was net mineralization of ammonium by MD1 (Fig. 3). We did not find a consistent relationship between the regeneration percentage and the substrate C/N ratio. Glycine, which has the lowest C/N ratio in our substrate series (ratio of 2), did cause the highest regeneration percentage, and asparagine, which has the same C/N ratio as glycine, ranked sixth, while leucine (ratio of 6) ranked third.

During the growth of MD1 on the organic nitrogen sources tested in this study, amino acids and BSA were consumed and ammonia and cell nitrogen were formed. The concentrations of amino acid and ammonia at the beginning and end of each experiment were measured. In heterotrophic aerobic bacteria, the main end products of growth on amino acids are cell material, CO_2, and ammonium (34). Since it is not very likely that MD1 produced any other nitrogen-containing compounds, it is possible to estimate the amount of nitrogen per cell at the end of each experiment. This amount was found to be 28.5 ± 8.9 fg of N per cell. To calculate carbon per cell from nitrogen per cell, the C/N ratio of the bacteria has to be known. For MD1, we measured a molar C/N ratio of 4.8 in carbon-limited continuous culture (19a). With this ratio, MD1 would have a carbon content of approximately 117.4 fg per cell.

Mixed cultures. Figure 4 shows the yields (cell number) of MD1 per micromole of N in the mixed cultures with either *E. huxleyi* (Fig. 4A) or *T. pseudonana* (Fig. 4B) after the growth of both algae and bacteria had ceased. With *T. pseudonana* as a partner, bacterium MD1 grew to higher densities in the mixed cultures (Fig. 4B, open bars) than in the pure cultures (Fig. 4B, hatched bars), even though equal amounts of organic N source were added. With *E. huxleyi*, however (Fig. 4A), the differences between pure and mixed cultures were small with some substrates and larger with others. Also, MD1 grew in the controls with ammonia as the N source, and remarkably, in the mixed cultures to which serine was added. This amino acid was not used by MD1 in pure culture.

In the mixed cultures with *T. pseudonana* (except with serine and aspartate), differences between the final cell numbers of MD1 in the mixed cultures and in pure culture were roughly comparable to the final cell number in the control mixed culture with ammonia as the sole N source.

In the mixed cultures with *E. huxleyi*, growth of MD1 was

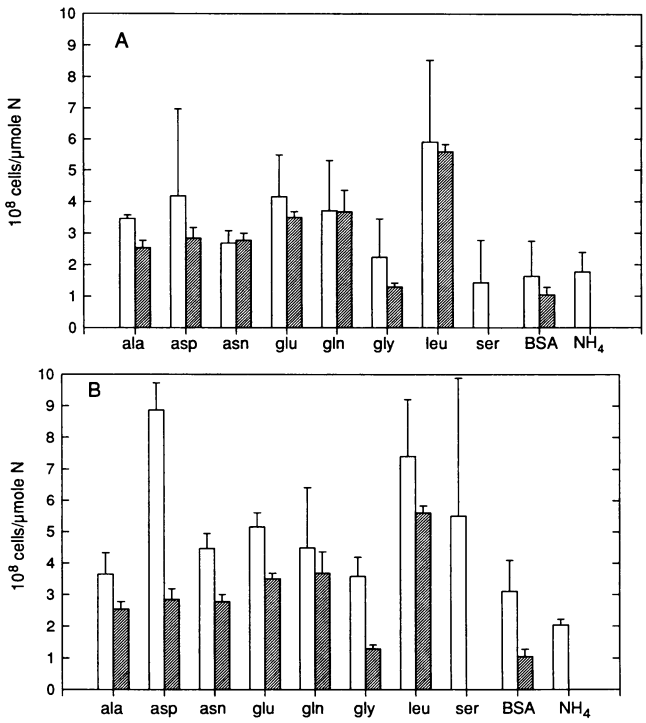

FIG. 4. Yields (cell number) of MD1 per micromole of amino acid N taken up in mixed culture (open bars) with *E. huxleyi* (A) and *T. pseudonana* (B). The yields in pure culture (hatched bars) are added for comparison. Bar heights represent means and error bars represent standard deviations of two separate experiments.

stimulated to a lesser extent and the cell number was even somewhat lower than the cell number in the control incubation with ammonium as the added N source. Again, MD1 showed growth in the mixed culture with serine, in contrast to the results for the pure culture of MD1.

Figure 5 shows the yields (cell number) of the algae in mixed culture with MD1. The cell numbers obtained for the axenic algal cultures with ammonium as the N source are added for comparison (Fig. 5, hatched bars). In all incubations, both species of algae grew with all N sources provided, even with the N compounds that did not support growth in the axenic culture (Fig. 1). This result suggests that MD1, by metabolizing BSA or the amino acids, was able to provide ammonia for algal growth, a situation most clearly observed for *T. pseudonana*, the species that could not use the organic N sources tested. While growth of *E. huxleyi* was stimulated in the incubations with Asp, Asn, Glu, Gln, Gly, and BSA, growth of the algae was considerably less in the mixed cultures on the N sources it could utilize in axenic culture (Ala, Leu, and NH_4^+). Final cell numbers of *E. huxleyi* and *T. pseudonana* in the control incubations with ammonia in the presence of MD1 reached 88 and 70%, respectively, of the cell numbers in the axenic controls.

DISCUSSION

Pure cultures. There are clear differences in the abilities of the two algal species to grow on organic nitrogen sources. *T. pseudonana* cannot grow on any of the substrates tested. *E. huxleyi* L shows fair to good growth on the neutral amino acids, such as leucine, alanine, and serine (Fig. 1). It has been shown that in algae, amino acids belonging to a certain group

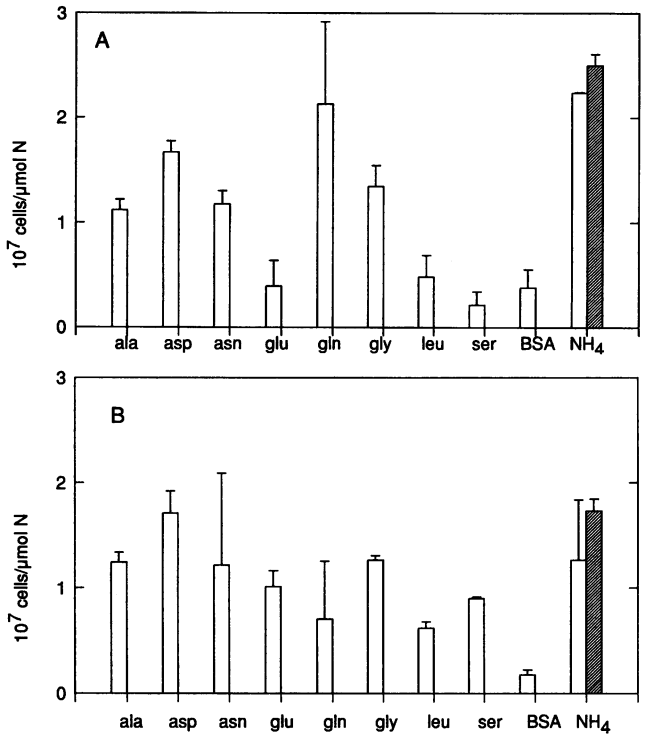

FIG. 5. Yields (cell number) of *E. huxleyi* (A) and *T. pseudonana* (B) in mixed culture with MD1 per micromole of amino acid N taken up. The yield of an axenic culture on ammonium is added for comparison (hatched bar at right). Bar heights represent means and error bars represent standard deviations of two separate experiments.

(neutral, basic, or acidic amino acids) are generally transported by the same transport system (2). Our results suggest that *E. huxleyi* L possesses an uptake system only for neutral amino acids. An alternative mechanism for algae to use amino acids for growth involves the selective uptake of the amino group through extracellular amino acid oxidases (31). We did not test our strains for the possession of such amino acid oxidases. However, Palenik and Morel (31) also tested a strain of *E. huxleyi* which did not exhibit amino acid oxidase activity. If our strain of *E. huxleyi* behaved similarly, then it only took up amino acids with normal transport systems, incorporating both nitrogen and carbon.

The ability of our strain, *E. huxleyi* L, to take up amino acids differs markedly from strains tested by Flynn (10). Flynn measured uptake over a 24-h period in two strains of different origin and came to the conclusion that both took up small amounts of a few nonneutral amino acids but did not take up the majority of the amino acids added. The difference between our results and those of Flynn is possibly caused by metabolic differences between the three strains involved. Another explanation is that in our study, amino acid concentrations were much higher than in the experiments described by Flynn, which possibly facilitated the amino acid transport.

MD1 was able to use the amino acids tested as growth substrates except for serine. This latter amino acid is not preferred by many bacterial strains that have been tested. Sepers (35) found that 48% of 66 aquatic bacterial isolates were able to grow on serine, while for instance 97% were capable of growth on aspartate and 91% were capable of growth on glutamate. It has been suggested that the amino acid concentrations in seawater reflect the preferences of the

TABLE 2. Cellular nitrogen contents calculated with different conversion factors[a]

N content (fg) per:		Reference
Cell	μm^3	
22.4	137	4
5.6		24
4.1	25	27
4.4	27	28
28.5	178	This study

[a] Values were calculated with conversion factors cited in the literature and this study. To calculate nitrogen per cell from nitrogen per unit volume, a mean cellular volume of 0.16 μm^3 is used. For this study, cellular nitrogen was calculated as described in Results.

organisms using the amino acids, with high residual concentrations reflecting low preference (11). This suggestion is supported by Braven et al. (5), who report an annual mean percentage of 12% of the total free amino acid concentration for serine in the English Channel, which is well above the average serine content in organisms.

MD1 grown in pure culture regenerated ammonium in all cases where growth occurred. This apparent surplus of substrate nitrogen suggests that MD1 was limited by carbon and/or energy. However, there is a large difference in cell yield per micromole of carbon between aspartate and asparagine and between glutamate and glutamine, which have equivalent carbon contents but different nitrogen contents (different by a factor of 2) suggesting nitrogen or energy limitation. The exact nature of the limitation was not determined by our experimental setup.

The amount of ammonium regenerated from a substrate has been reported to be related more or less linearly to the substrate C/N ratio (15, 16). We did not find such a relationship between the regeneration percentage and the substrate C/N ratio (Fig. 3). This inconsistent pattern could be the result of us using a single bacterial isolate, with its particular metabolic properties. In contrast, natural bacterial assemblages were used in the studies mentioned. The presence of many different types of bacteria probably results in the canceling out of the effects of any substrate preferences by the individual bacterial species.

Growth yield, substrate C/N ratio, and organism C/N ratio together determine whether nitrogen is regenerated as ammonium or not (15). We therefore attempted to calculate some of these values from our data. We estimated a nitrogen content of 28.5 fg per cell. This value is high compared with values calculated from conversion factors (Table 2) and is best approximated by the conversion factor reported by Bratbak (4). The high nitrogen content of MD1 is not caused by a difference in the size of the MD1 cells compared with the size of the bacteria used in the references cited in Table 2. MD1 had a mean volume of 0.16 μm^3 at the end of the growth phase, which is well within the range found for marine bacteria (4, 27, 28).

From the estimate of the nitrogen content of MD1 and its C/N ratio, we calculated a cellular carbon content of 117 fg per cell. If we use this value to calculate a carbon growth yield, we find a mean yield of 68% for the amino acids with one nitrogen atom. The two amino acids with two nitrogen atoms have calculated yields above 100% (asparagine, 170%; glutamine, 139%). The bacterial C/N ratio we used to calculate the yields was most probably not valid for these cultures. This ratio can vary with the physiological state of the bacteria (4, 27, 28). For

nitrogen, the mean molar growth yield is 56% (range, 28 to 99%).

Mixed cultures. The cell yields of both algae (Fig. 5) are influenced in mixed culture by the regeneration of ammonium by the bacteria. Since *T. pseudonana* is unable to grow on amino acids (Fig. 1), it is clear that all growth observed in mixed culture was due to bacterial regeneration of ammonium. It is possible that *E. huxleyi* used amino acids in some cases. However, MD1 depleted all amino acids within 6 days, whereas *E. huxleyi* grew very slowly on amino acids. In the competition for amino acids with *E. huxleyi*, MD1 probably took up most of the substrate before *E. huxleyi* could do so. It is therefore reasonable to conclude that ammonium was the main nitrogen source for both algae. Assuming that this is the case and excluding any influences of alga-bacterium interaction on the regeneration rates, the growth yields of *E. huxleyi* and *T. pseudonana* on the different amino acids should reflect the regeneration percentages found in the pure cultures of MD1, with glycine giving the highest algal yields and BSA giving the lowest algal yields. They do not. Dissolved organic carbon, produced by the algae and used by the bacteria, probably influenced the amount of ammonium regenerated by the bacteria.

For the bacteria, the cell numbers in mixed culture are in most cases higher than in pure culture, although the magnitudes differ from one species to the other. Although the difference is small in some cases (for instance, Leu and Asp [Fig. 4A]), there is a strong suggestion that the amino acids as growth substrates are supplemented by excretion products produced by the algae. In the mixed cultures with *E. huxleyi* (Fig. 4A), the increase in cell yield is small in some cases and greater in others. This result could be caused by a certain amount of amino acid uptake by *E. huxleyi*. The exact nature of the substrates taken up by the algae could not be determined with our experimental setup. Successful competition of algae with bacteria for amino acid uptake has been shown previously (33).

In mixed culture with *T. pseudonana* (Fig. 4B), the increase in yield appears to be surprisingly constant. The magnitude of the increase is approximately equal to the yield attained in a mixed culture with NH_4^+ as the nitrogen source, with all carbon substrate being produced by the algae. The two exceptions are serine and aspartate. For serine, no growth of MD1 was observed in pure culture, but in mixed culture, the yield on serine was comparable to that on leucine. This difference can be explained by some form of cometabolism, whereby excretion products from the algae, taken up together with serine, enable MD1 to use serine as a substrate. It should be noted, however, that the growth of both *T. pseudonana* and MD1 in the mixed culture on serine was sluggish and that at the end of the experiment serine was not depleted. This is not expressed by the bar in Fig. 4B, as it shows the yield per micromole of substrate actually taken up. The poor growth of the algae probably resulted in a relatively large amount of organic carbon being excreted. For aspartate, all the substrate was used and both algae and bacteria showed good growth. The cause of the stimulation of bacterial cell yield is not clear here.

Ecological implications. This study shows that remineralization can be an important source of nitrogen for phytoplankton when DFAA or DCAA are both nitrogen and carbon sources. Although initial amino acid concentrations as used here were of the same order as the spring concentrations of inorganic nitrogen in the field, the concentrations were much higher than natural organic nitrogen concentrations (for a review, see reference 2). It is well-known that under nitrogen-limiting conditions, algae are better capable of utilizing alternative nitrogen sources (12, 29). Also, algae are known to increase excretion when stressed. To assess the effects of alga-bacterium interactions under nutrient-limited conditions, nitrogen-limited continuous cultures should be used to investigate the behavior of the mixed culture with amino acids as the sole nitrogen source.

Although in our experiments no clear effect of the substrate C/N ratio on bacterial growth and regeneration was obvious, this ratio will certainly affect the nutritional status of bacteria in the pelagic ecosystem. Some researchers have argued that natural bacterial assemblages may need supplemental ammonium for growth (15, 37). Other studies show that regeneration of nitrogen by bacteria is a common feature of natural systems (7, 23, 39) and even that ammonium regeneration and assimilation by bacteria can occur simultaneously (37). Our results show that organic carbon produced by phytoplankton can influence the amount of nitrogen regenerated by bacteria, but this does not mean that by doing so, the phytoplankton causes its own demise by stimulating bacterial ammonium uptake (3). Even in our simple two-species cultures, neither bacterial regeneration nor algal excretion could be correlated to substrate C/N ratio or bacterial growth yield. To assess the importance of these processes in the field, more information is needed on the nutritional status of bacteria in the field, as well as on their growth yields, especially when grown on complex substrates. This information, together with data on the nature and production rates of dissolved organic carbon by phytoplankton, will supply insight in the role of interactions between algae and bacteria in pelagic ecosystems.

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