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Nutrient regulation of bacterial production and ectoenzyme activities in the subtropical North Pacific Ocean

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Abstract

Interactions between *Bacteria* and dissolved organic matter (DOM) in the open ocean are poorly understood. While it is likely that particular compounds may disproportionately regulate heterotrophic activity, very little is known about the underlying processes. Through 10 cruises between December 1996 and April 1998 we investigated how heterotrophic (non-pigmented) *Bacteria* cell production, per cell α- and β-glucosidase and leucine aminopeptidase (LAPase) activities, and ^{14}C-glucose uptake in 0.8 μm filtered seawater (fsw) cultures at Station ALOHA (22°45′N, 158°W) responded to organic and inorganic nutrient additions (glucose, single amino acids, NH_4^+, NO_3^-). Bacterial cell production did not change significantly in fsw with glucose (1 μM) or single exogenous N sources (1 μM N) compared to that in fsw alone. Furthermore, there was no significant difference in heterotrophic bacterial cell production in fsw amended with organic or inorganic N, nor between that in fsw with organic N and glucose, or inorganic N and glucose. Cell production did increase significantly, however, in fsw with exogenous glucose (0.38 μM) plus 1 μM inorganic N (NH_4^+) relative to that in fsw only, in fsw with glucose, and in fsw with 1 μM N as amino acids (His, Tyr, Leu). There was no significant difference between heterotrophic bacterial cell production in fsw with glucose, glucose plus amino acids, and that in fsw alone. Cell-specific LAPase activity increased significantly relative to that in unamended fsw when exogenous glucose plus NH_4^+ or NO_3^- were provided, but amino acids, glucose, NH_4^+ or NO_3^- alone had little or no effect. α-Glucosidase activity tended to increase with exogenous His and Tyr additions. Our results suggest that heterotrophic activity at Station ALOHA can be regulated by the abundance of particular compounds, regardless of their total concentrations. It appears that auxotrophy and de novo synthesis of cell protein from glucose may coexist among *Bacteria* at Station ALOHA, and that regulation of ectoenzyme expression is independent of product availability.

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1. Introduction

Dissolved organic matter represents the vast majority of reduced carbon in the oceans, and both its chemical composition and its availability to organisms are poorly understood (Hedges and Lee, 1993). *Bacteria*, both heterotrophic and photoautotrophic, dominate community metabolism and biogeochemical cycling in hyperoligotrophic ecosystems like the subtropical North Pacific gyre (e.g. Campbell et al., 1994; Karl and Dobbs, 1998). Stoichiometric approaches to bacterial growth give little information on how individual substrates affect growth, since compounds containing equivalent amounts of C, N and P do not affect bacteria in the same way (Christian and Karl, 1998). Moreover, short-term studies of bacterial production and growth under various inorganic nutrient, dissolved organic matter (DOM) or temperature regimes overlook temporal changes in community structure (e.g. Le et al., 1994; Middleboe et al., 1995).

The DOM pools in most aquatic environments are complex mixtures of compounds with variable molecular weight and bioavailability. Indeed, the major part of the DOM pool in surface seawater in the subtropical North Pacific is uncharacterized. The presence of cell-surface-associated enzymes, termed ectoenzymes, allows bacteria to hydrolyze DOM polymers to monomeric constituents that can then be assimilated. Activities of the ectoenzymes α- and β-glucosidase and leucine aminopeptidase in different habitats have been described (Chróst, 1991; Christian and Karl, 1995a; Karner and Rassoulzadegan, 1995). It should be borne in mind, however, that these ectoenzymes are not expressed by all bacteria (Zdanowski and Donachie, 1993; Martinez et al., 1996), nor are they exclusively bacterial in origin; α-glucosidase and aminopeptidase also are produced by certain phagotrophic nanoflagellates (Gonzalez et al., 1993; Karner et al., 1994) and marine yeast (Donachie, 1995), and might be expressed by planktonic *Archaea*, and β-glucosidase activity has been reported in flagellates (Meyer, 1976). Furthermore, the pattern of enzyme activities changes along with the microbial community in response to both biotic and abiotic factors. For example, enzyme activity associated with particles is prodigious (Smith et al., 1992) and that on particles derived from the collapse of a phytoplankton bloom may account for seasonal changes in β-glucosidase activities (Chróst, 1989).

In waters west of the Antarctic Peninsula, the ratio of protease to β-glucosidase activities varies seasonally with changes in ecosystem productivity (Christian and Karl, 1995a). Bacterioplankton ectoaminopeptidase activities are regulated by specific compounds in the environment, e.g. histidine (His) and phenylalanine (Phe), but exogenous N compounds have little effect on α- or β-glucosidase activities in waters west of the Antarctic Peninsula (Christian and Karl, 1998). There are no comparable data for other marine ecosystems. The establishment of the US-JGOFS Hawaii Ocean Time-series (HOT) Station ALOHA (A Long Term Oligotrophic Habitat Assessment) north of Hawaii (Karl and Lukas, 1996) provided an unique research opportunity to investigate regulation of microbial ectoenzyme activities in the subtropical North Pacific Ocean.

Mean seawater temperature in the upper 50 m at Station ALOHA shows little seasonal variation (22.5–27.5°C, $\sim$ 10 yr data set covering 88 HOT cruises). Insolation at Station ALOHA is high, with daily photosynthetically available radiation (PAR, 400–700 nm) during HOT cruises 1–50 showing a monthly average of 26.7–52.4 mol quanta $m^{-2}\,day^{-1}$ (Karl et al., 1996). Surface NO_3^- concentrations here are low (< 10 nM) and food webs relatively complex; phytoplankton tend to be small and numerically dominated by *Prochlorococcus* and *Synechococcus* (Campbell et al., 1997)

and microorganisms both produce and consume most of the primary production. Heterotrophic metabolism at this site relies upon autochthonous, photosynthetically derived organic matter.

Both biosynthesis and catabolism of amino acids are of interest from a biogeochemical standpoint because, while there is a clear energetic advantage to utilizing amino acids in preference to de novo synthesis from simple substrates like glucose or acetate, not all *Bacteria* possess the biochemical pathways to do so (Guirard and Snell, 1962). Even though the mixture of amino acids present in nature might differ from that in cell protein, it is likely that even a small energetic advantage would be favorable. In this respect, nitrogen in protein satisfies 20–65% of the bacterial N demand for growth in the northern Sargasso Sea, but other nitrogen sources are still required (Keil and Kirchman, 1999).

We investigated the physiological diversity and production of *Bacteria* at Station ALOHA with a series of seawater culture experiments designed to determine responses to stoichiometrically equivalent additions of C and N in different chemical forms. We estimated cell production of heterotrophic (non-pigmented) *Bacteria* as well as *Prochlorococcus* and *Synechococcus* spp., expression of hydrolytic ectoenzymes (α- and β-glucosidase, leucine aminopeptidase) and uptake of radiolabelled glucose in seawater filtered through 0.8 μm membrane filters to remove protozoan grazers. We report here that, at Station ALOHA, inorganic nitrogen plus glucose generally stimulates bacterial production more than individual amino acids, and whether the inorganic nitrogen is in the form of ammonium or nitrate is relatively unimportant. These results indicate that the abundance of specific compounds regulates overall osmoheterotrophic activity independently of the total concentrations of presumably labile forms of C and N, and that heterotrophic growth of *Bacteria* is neither strictly nitrogen nor energy limited.

2. Materials and methods

2.1. Sample site

All experiments described in this paper were conducted during 10 cruises to Station ALOHA (Karl and Lukas, 1996) in the subtropical North Pacific Ocean between December 1996 (HOT-78) and April 1998 (HOT-92) (Table 1).

2.2. Nutrient supplements

Seawater collected with a Go-Flo® bottle (General Oceanics, Miami) at a depth of 45 m was filtered through 0.8 μm Nuclepore polycarbonate filters. Filters were pre-washed immediately beforehand to remove organics by filtering ca. 500 ml of distilled water. Filtration also removed grazers from the seawater. The water was then dispensed into acid-washed and double-distilled (dd) H_2O-rinsed 500 ml polycarbonate (PC) bottles. Amino acids as sole exogenous N sources were added to a final concentration of 1 μM N. Glucose as a single exogenous nutrient was added to a final concentration of 1 μM glucose (6 μM C). When glucose and single exogenous N sources were both added to the same bottle, their final concentrations were 0.38 μM glucose (2.28 μM C) and 1 μM N, respectively. A stock solution of each supplement was prepared in dd H_2O in acid washed and dd H_2O rinsed glassware immediately before each cruise. NH_4^+ was provided as NH_4Cl, and

Table 1
Chronology of cruise and experiment dates, substrates applied and experimental analyses

Cruise	Date	Substrates	Experiments
78	10 December 1996	Glucose, His, Tyr, Phe, Leu, Pro, Trp, NH_4^+, NO_3^-	Ectoenzymes, cytometry
81	11 March 1997	Glucose, His, Tyr, Phe, Leu, Pro, Trp, Gly, NH_4^+, NO_3^-	Ectoenzymes, cytometry
82	8 April 1997	Glucose, His, Tyr, Phe, Leu, Pro, Trp, Gly, NH_4^+, NO_3^-	Ectoenzymes, cytometry
83	6 May 1997	Glucose, His, Tyr, NH_4^+, glu/His, glu/Tyr, glu/NH_4^+	Ectoenzymes, cytometry, ^{14}C-glucose uptake (0.8 μm fraction)
84	3 June 1997	Glucose, His, Tyr, NH_4^+, NO_3^-, glu/His, glu/NH_4^+, glu/NO_3^-, glu/Leu	Ectoenzymes, cytometry, ^{14}C-glucose uptake (0.8 μm fraction)
85	4 July 1997	Glucose, glu/His, glu/Tyr, glu/NH_4^+, glu/NO_3^-, glu/Leu	Ectoenzymes, cytometry, ^{14}C-glucose uptake (0.8 μm fraction)
86	1 August 1997	Glucose, glu/NH_4^+, glu/NO_3^-, glu/Leu	Ectoenzymes, cytometry, ^{14}C-glucose uptake (0.4 μm fraction)
87	25 August 1997	His, Tyr, NH_4^+, NO_3^-, mixed amino acids	Ectoenzymes, cytometry
88	4 December 1997	His, Tyr, Leu, NH_4^+, NO_3^-, mixed amino acids	Ectoenzyme (LAPase only), cytometry
92	14 April 1998	Glucose, His, Tyr, Phe, NH_4^+, NO_3^-	Ectoenzymes, cytometry

NO_3^- as KNO_3, both analytical grade (Fisher Chemicals, New Jersey). During HOT-78, 81 and 82, single bottles per treatment were used in order that a broad range of substrates be tested. A smaller range of substrates was considered in duplicate or triplicate PC bottles from HOT-83 onwards. Control bottles contained unamended 0.8 μm filtered seawater (fsw). All PC bottles were incubated for 24 or 48 h in darkness at surface seawater temperature.

2.3. Cell numbers

Immediately after nutrient additions, a 1 ml subsample from each PC bottle was fixed in a sterile cryovial with 0.2 μm filtered glutaraldehyde added to a final concentration of 0.2%. The samples were immediately placed into liquid nitrogen for 3 days, and thereafter stored at -20°C. Numbers of non-pigmented *Bacteria* plus *Archaea* (hereafter referred to as heterotrophic bacteria), *Prochlorococcus*, *Synechococcus* and eukaryotic picophytoplankton (picoeukaryotes) in these samples were determined by flow cytometry (Monger and Landry, 1993; Campbell et al., 1994). Additional samples were withdrawn from each PC bottle at the end of the incubation for enumeration by flow cytometry. Cell production of each population is described on the basis of changes in cell numbers over the period of the incubation.

2.4. Ectoenzyme activities

Potential α- and β-glucosidase (EC 3.2.1.20 and EC 3.2.1.21, respectively) and leucine aminopeptidase (EC 3.4.1.1) (LAPase) activities in each PC bottle were determined as described by Christian

and Karl (1995a,b) after Hoppe (1983) and Somville and Billen (1983), immediately after samples for cell numbers were taken; the enzyme activities determined were considered potential activities because substrate analogs were applied at experimentally derived saturating concentrations. LAPase activities are presented on a per prokaryotic cell (sum of *Bacteria* plus *Archaea*) basis due to uncertainty about the contribution, if any, to this activity from *Prochlorococcus* and *Synechococcus* in waters at Station ALOHA. LAPase activity has been reported in cultures of *Synechococcus* (Martinez and Azam, 1993).

Glucosidic enzyme activities are described on a per heterotrophic bacterial cell basis. We expect photoautotrophs to manufacture the sugars they require from inorganic carbon through photosynthesis, and thus be unlikely to need glucosidic ectoenzymes to derive sugars from the hydrolysis of DOC in the environment. Inclusion of pigmented *Bacteria* would decrease the cell-specific rates by ca. 5–33%. Activities of α- and β-glucosidase were calculated from the fluorescence measurements of duplicate or triplicate 6 ml samples from each PC bottle, with an additional, time-zero control sample denatured immediately by addition of 100 µl of saturated mercuric chloride solution (final concentration, 4 mM). Fluorescent substrate analogs, 4-methylumbelliferyl-α-D-glucoside or 4-methylumbelliferyl-β-D-glucoside, were added at 1.6 µM to the respective vials. Time-zero samples were immediately placed in a freezer (-20°C).

Leucine aminopeptidase activities were calculated for duplicate 6 ml samples in sterile polypropylene centrifuge tubes with L-leucine β-naphthylamide at saturating concentration (1 mM), again with mercuric chloride denatured time-zero samples placed in a freezer immediately after inoculation. After incubation, 100 µl of saturated mercuric chloride solution was added to each sample and the denatured samples immediately placed in a -20°C freezer. Sample fluorescence was determined in a Perkin-Elmer LS-5 spectrofluorometer against a 4-methylumbelliferone standard in deep seawater ($\sim$4850 m) from Station ALOHA at $\lambda_{ex} = 360$ nm and $\lambda_{em} = 448$ nm, or a β-naphthylamine standard in seawater at $\lambda_{ex} = 338$ nm and $\lambda_{em} = 407$ nm.

To determine if heterotrophic bacteria smaller than 0.4 µm respond to glucose and N supplements through changes in cell numbers and/or ectoenzyme activities, we measured each of these parameters in 0.4 µm filtered seawater containing 1 µM exogenous N and 0.38 µM exogenous glucose during HOT-86. This approach facilitated the description of these activities in the absence of larger *Bacteria* such as *Prochlorococcus* and *Synechococcus*, which were not present in the 0.4 µm filtrate.

2.5. Uptake of ^{14}C-glucose

During HOT-83 to HOT-86 inclusive, microbial uptake of D-[^{14}C(U)]-glucose in the presence of N supplements (1 µM-N) in 0.8 µm-filtered seawater was followed at approximately 8-h intervals through $\sim$24 h dark incubations at in situ surface temperatures. D-[^{14}C(U)]-glucose was added at 185 Bq ml^{-1}, equivalent to a final glucose concentration of 0.38 µM. Radioactivity collected onto microfine glass fiber filters (Whatman GF/F) was determined by liquid scintillation counting. During HOT-83, 84 and 85, cell numbers and enzyme activities were followed over the same time course in parallel incubations with unlabeled glucose at 0.38 µM. On HOT-86, cell numbers and enzyme activities were followed only in the <0.4 µm fraction over a 24 h incubation.

3. Results

3.1. Effects of nutrient supplements on cell production

Cell production rates of the heterotrophic bacteria, *Prochlorococcus*, *Synechococcus* and picoeukaryotes were determined in dark-incubated filtered seawater cultures amended with organic or inorganic N and/or glucose. Considering the data for all cruises, no single exogenous supplement significantly affected the cell production rate of any of these populations compared to that in parallel fsw only (log-transformed data, regular or paired *t*-test, and nonparametric (Wilcoxon) equivalents where sample size allowed) through 24 or 48 h incubations. Moreover, the mean production rate calculated for all cultures with exogenous N or glucose did not significantly differ from that in fsw controls. Heterotrophic bacteria cell production rates tended to be higher in fsw with exogenous nutrients than in unamended fsw (Fig. 1). *Prochlorococcus, Synechococcus* and picoeukaryote cell production rates in fsw with exogenous N and/or glucose throughout the study did not differ significantly from those in fsw alone.

During HOT-83 to HOT-86, heterotrophic bacterial cell production in fsw, with inorganic N (pooled NH_4^+ and NO_3^- treatments) plus glucose was significantly greater than in unamended fsw controls (two-tailed *t*-test, $p < 0.025$), in fsw with glucose only ($p < 0.05$), and in fsw with organic N (pooled amino acid treatments) plus glucose ($p < 0.05$). There was no significant difference between heterotrophic bacteria cell production rates in fsw with glucose plus organic N, with glucose only, and in fsw controls (Fig. 2).

Heterotrophic bacteria cell production rates in fsw with glucose plus inorganic N differed with each N source. Production rates in fsw with glucose plus NH_4^+ significantly exceeded that in fsw with glucose only (*t*-test, $p < 0.025$) and in unamended fsw ($p < 0.025$). There was no significant difference between production rates in fsw with NO_3^- plus glucose, and those in fsw with glucose

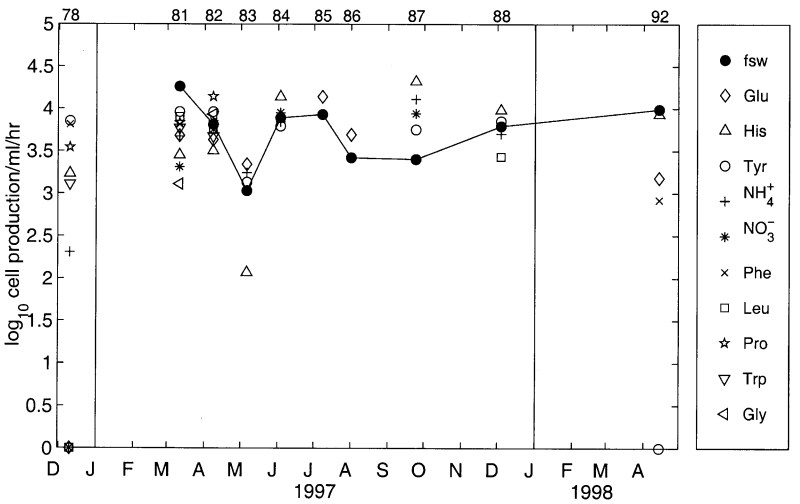

Fig. 1. Cell production rates ($\log_{10}$ cells/ml/h) of heterotrophic bacteria in filtered seawater with and without single exogenous N supplements, or glucose. Numbers along the top of the graph refer to the cruise number.

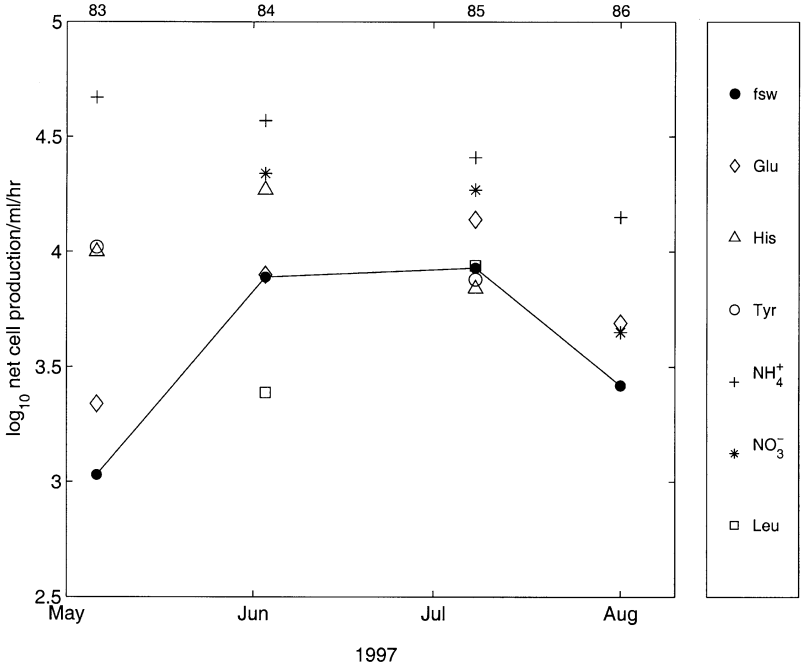

Fig. 2. Cell production rates ($\log_{10}$ cells/ml/h) of heterotrophic bacteria in filtered seawater with exogenous N and/or glucose. Numbers along the top of the graph refer to the cruise number.

only, or in unamended fsw. We should point out, however, that production rates with glucose and NO_3^- were considerably higher than in fsw or with glucose alone, but there are too few data to verify this statistically. The mean difference between production rates in fsw with glucose plus NH_4^+ or with glucose plus NO_3^-, however, did not differ significantly.

3.2. Ectoenzyme activities

Throughout the study, α-glucosidase activities in the presence of His or Tyr were, respectively, 210 and 260% those in fsw, but these increases were not statistically significant, likely because of high measurement variability. Although α-glucosidase activity was significantly higher in the presence of Pro and Trp ($p < 0.02$, two-tailed t-test, log-transformed data, $n = 3$), these compounds were assayed only on the first 3 cruises. During these cruises, α-glucosidase activity in the presence of most exogenous nutrient sources was higher than in fsw (Fig. 3a). The extent to which this pattern might represent a change in community structure is unknown. During the same three cruises, mean α-glucosidase activities in fsw with exogenous glucose significantly exceeded those in unamended fsw ($p < 0.01$, two-tailed t-test, log transformed data) and in fsw with amino acids ($p < 0.05$), but not those in fsw with inorganic N. β-Glucosidase activities were significantly higher only in the presence of glucose ($p < 0.02$) (Fig. 3b). Per cell LAPase activities did not change significantly in the presence of single exogenous nutrients when compared to activity in fsw only (Fig. 3c). LAPase activity in unamended fsw displayed a different temporal pattern to those of the glucosidases in the same fsw.

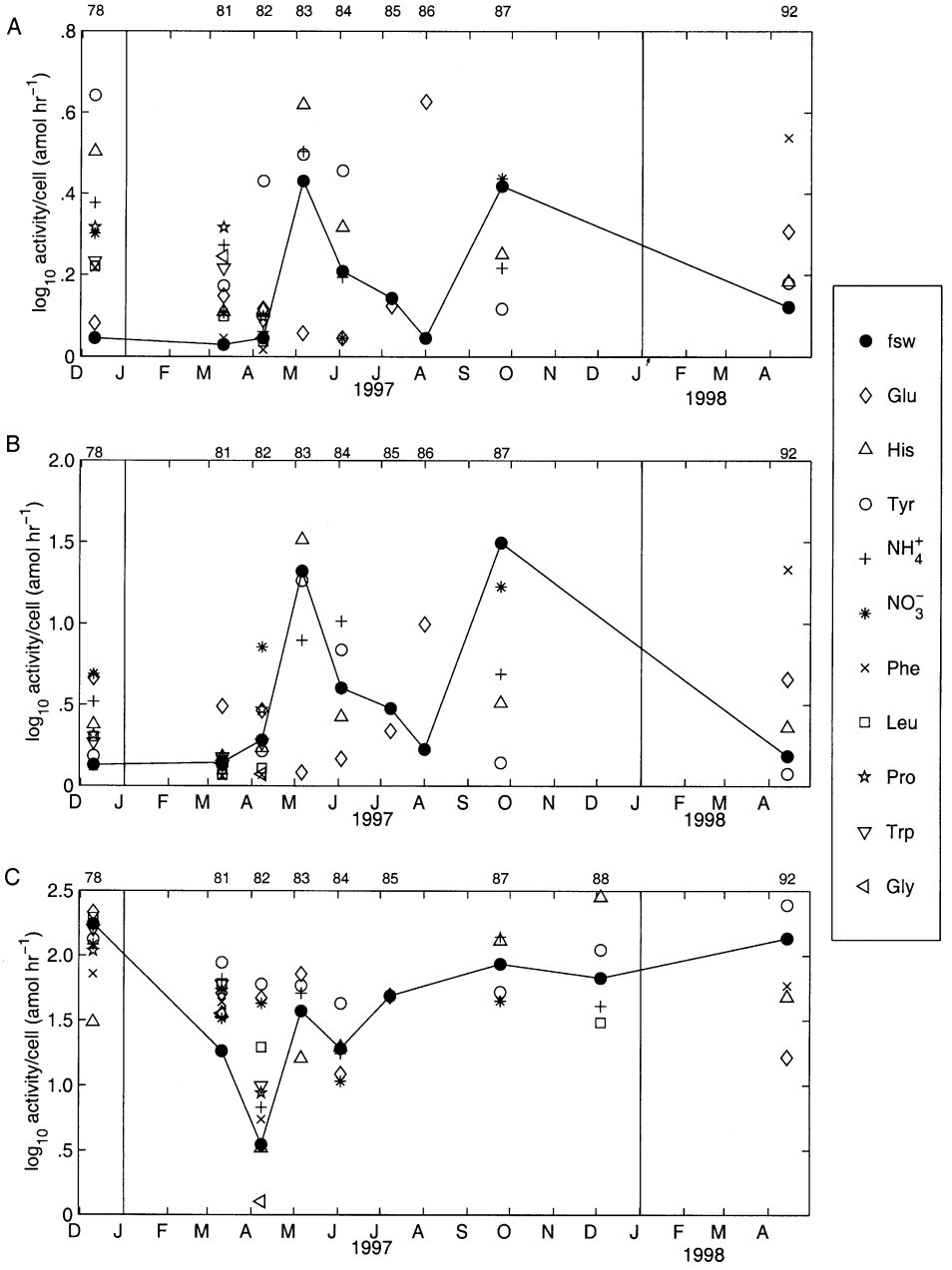

Fig. 3. (A) Cell-specific α-glucosidase and (B) β-glucosidase activities of heterotrophic bacteria, and (C) LAPase activities per prokaryote in filtered seawater with exogenous N or glucose. Numbers along the top of the graph refer to the cruise number. Activities are presented as $\log_{10}((\text{amol/cell/h}) + 1)$.

Cell-specific α- and β-glucosidase activities in fsw with exogenous glucose plus N did not differ significantly from those in fsw with glucose only (Fig. 4). Mean cell-specific LAPase activity in fsw with glucose plus NH_4^+ was 420% that in the unamended fsw, and 260% that in fsw with glucose

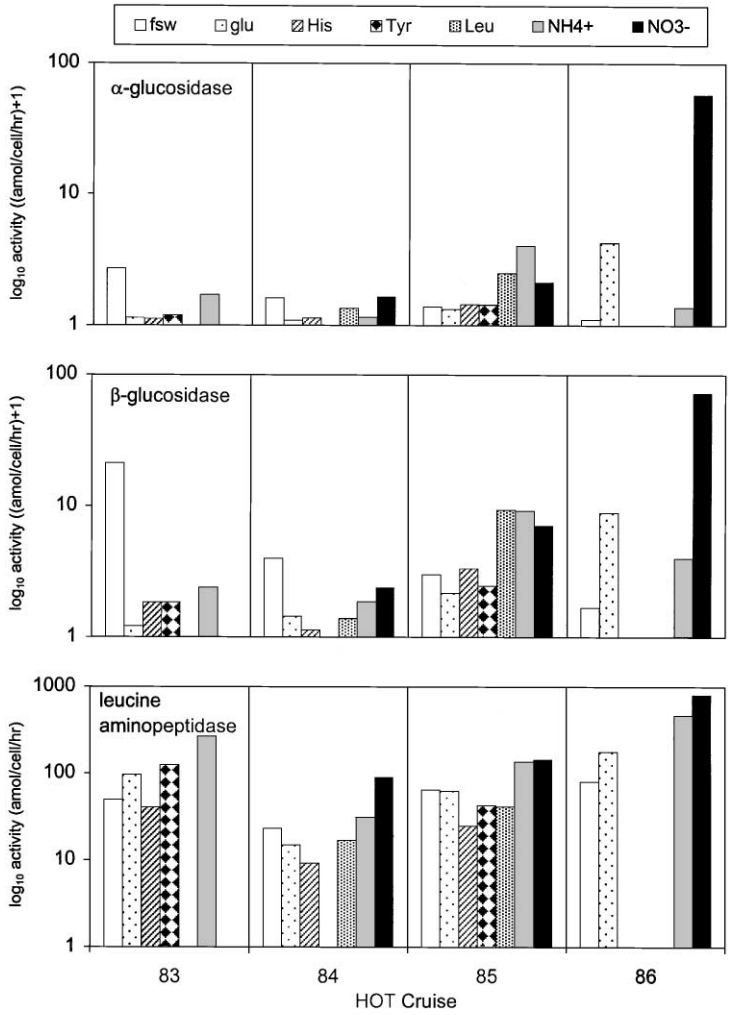

Fig. 4. Cell-specific α- and β-glucosidase and LAPase activities of heterotrophic bacteria in filtered seawater with single exogenous N sources and/or glucose. LAPase activities are presented for the heterotrophic bacteria to facilitate comparison between HOT-86 during which only these cells were present in the culture, and HOT-83 to HOT-85 when other prokaryotes were present. Enzyme activities of the heterotrophic bacteria and prokaryotes during HOT-83 to HOT-85 responded identically to any particular substrate.

only (Fig. 4). Respective activity increases in fsw with glucose plus NO_3^- were 640 and 400%. Due to the small number of data in each category, however, these increases are not statistically significant. When considered relative to the aggregate of the control, glucose and amino acid treatments (which did not differ significantly), LAPase activity in fsw with glucose plus NH_4^+ was 394% of that in the control group ($p < 0.005$, two-tailed t-test). LAPase activity in fsw with glucose plus NO_3^- was 600% of that in the control group ($p < 0.01$). Highest activities of each enzyme were recorded in the 0.4 μm filtrate with glucose plus NO_3^- during HOT-86.

3.3. Glucose uptake rates

Glucose uptake rates in fsw with and without exogenous N were nonlinear through ~ 24 h incubations and were generally characterized by a lag phase of 7–11 h (Fig. 5). With few exceptions, the lag phase was followed by elevated uptake rates determined by the exogenous N source. Highest glucose uptake rates were consistently found with NH_4^+ (740% that in glucose only controls) followed by NO_3^- (640% that in controls) and exogenous amino acids (190% that in controls). Glucose uptake rates with amino acids were not significantly greater than in controls.

4. Discussion

In this study we investigated the effects of exogenous organic and inorganic nutrients on the cell production rates of heterotrophic bacteria, *Prochlorococcus*, *Synechococcus* and picoeukaryotes, and on per cell ectoenzyme activities and glucose uptake in seawater cultures in the subtropical North Pacific Ocean. Our data show that the heterotrophic bacteria responded differently in the above terms to exogenous nutrients, but that no single exogenous nutrient consistently affected any of these metabolic parameters throughout the study. We also demonstrated that *Prochlorococcus, Synechococcus* and picoeukaryote cell production rates were not enhanced in the presence of exogenous N and/or glucose.

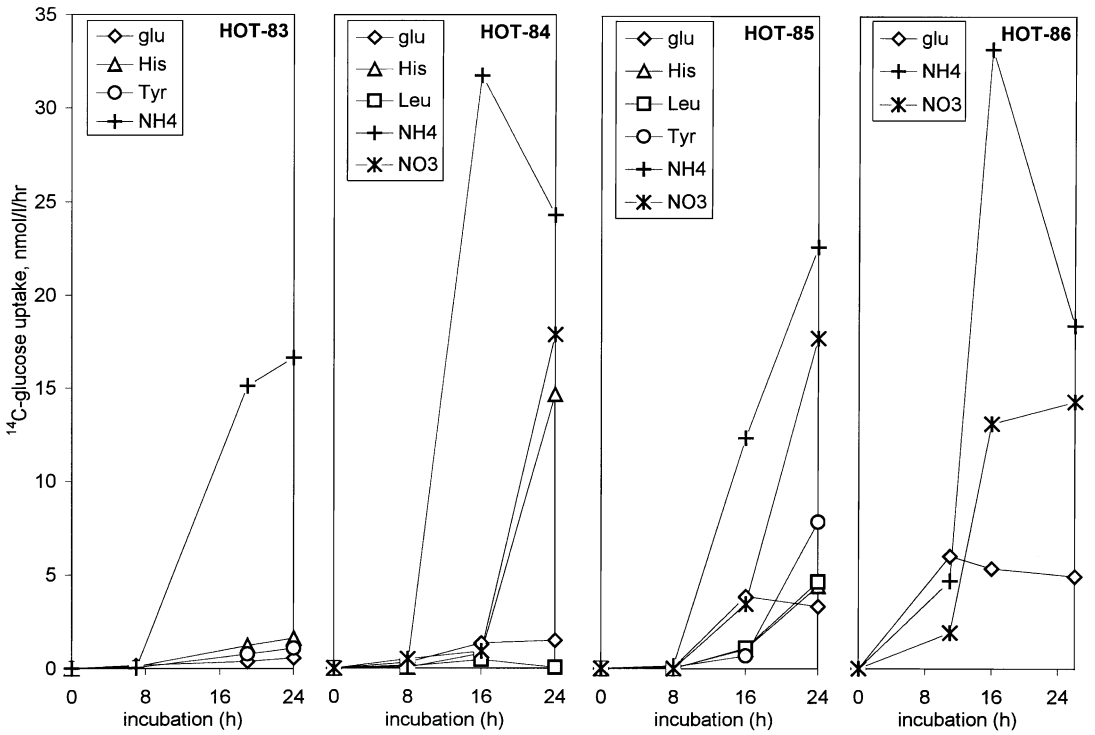

Fig. 5. Uptake rates of D-[^{14}C(U)]-glucose in the presence of exogenous N in filtered seawater.

Some amino acids, particularly His and Phe, have been shown to regulate LAPase activities in the Southern Ocean (Christian and Karl, 1998). Over the duration of our study at Station ALOHA, however, we found no significant regulation of glucosidase or LAPase activities in fsw with single exogenous nutrients, including His and Tyr. Different responses to the same nutrient between cruises in terms of cell production or ectoenzyme activities may reflect physiological changes in the population or changes in community structure. Such changes are reflected in seasonal variations in patterns of substrate utilization by bacterioplankton (Zdanowski and Figueiras, 1999) and in varying enzyme activities (Rehnstam et al., 1993). Cell production rates and LAPase activities of the heterotrophic bacteria consistently increased in fsw with exogenous glucose plus inorganic N. Furthermore, inorganic N significantly enhanced glucose uptake rates in fsw over those in 0.8 μm fsw with glucose only or with amino acids. Griffiths et al. (1984) considered that both the quality and quantity of the organic nutrient pool would regulate bacterial growth as higher diversity and quantity satisfy the growth requirements of a greater fraction of the population. The frequent enhancement of growth rates by single nutrient additions in this paper suggests the nutrient pool would otherwise have satisfied growth requirements. In this respect, Tyr was the exogenous nutrient that when applied alone to fsw in our work, most commonly stimulated cell production compared to that in unamended fsw.

At the Bermuda Atlantic Time-series Study (BATS) site, bacterial cell production was enhanced by addition of glucose only and *Bacteria* were considered generally energy limited (Carlson and Ducklow, 1996). The same authors noted that mixed amino acids stimulated microbial growth to the same degree as glucose, but the mixed organics and inorganics in an algal lysate stimulated bacterial production beyond that of the control, glucose or mixed amino acids, suggesting multiple compounds maximized bacterioplankton production. Similarly, Hagström et al. (1984) showed that increases in bacterial biomass could be entirely accounted for through removal of DOM from fsw. By consistently recording the highest cell production rates of the heterotrophic bacteria when NH_4^+ and glucose were offered together, our results suggest that *Bacteria* at Station ALOHA were limited by both C and N availability. We found that exogenous NH_4^+ alone did not enhance bacterial numbers over those with single amino acids, contrasts with the findings of Horrigan et al. (1988) that either NH_4^+ or NO_3^- alone enhanced heterotrophic bacterial growth over DOM. Alternatively, the extant microbial populations at Station ALOHA could be adapted to the prevailing C : N ratio of the DOM pool. The addition of only one substrate deficient in either C or N would therefore confer little advantage, an assumption supported by the constraints on bacterial production in the eastern North Pacific by the substrate DOC:DON ratio (Cherrier et al., 1996). Although the small sample size limits our ability to demonstrate a significant effect of NO_3^-, the balance of evidence does suggest either NH_4^+ or NO_3^- plus glucose significantly enhances cell production rates. Glucose-derived carbon might be used for cell growth, as indicated by high rates of ^{14}C-glucose uptake in the presence of NH_4^+ and the pronounced increases in cell numbers with this mixture. Glucose uptake at Station ALOHA is regulated by N species, with inorganic N (NH_4^+ or NO_3^-) consistently promoting higher uptake rates than amino acids. Catabolism of amino acids at Station ALOHA is not a viable alternative source of N for bacteria, and the breakdown of His, Tyr or Leu to NH_4^+ likely proceeds slowly.

Rath et al. (1993) described an inverse relationship between LAPase activity and bacterial production at oligotrophic stations in the Caribbean Sea, and Münster (1991) invoked nutrient limitation to account for high LAPase activities in a lake with low dissolved amino acid

concentrations. In contrast, we found no significant correlation between LAPase activities and cell production rates in seawater at Station ALOHA.

We do not expect N supplements to enhance glucosidase activities directly but elevated glucosidase activities in the presence of most N substrates during this work likely reflect other aspects of cell metabolism stimulated by the supplement. It is also possible that the additional N is required to produce glucosidic enzymes. Proteolytic enzyme activity in marine habitats is not solely of heterotrophic bacterial origin and may be derived from photosynthetic *Bacteria* such as *Synechococcus* (Martinez and Azam, 1993) and *Eucarya* such as phagotrophic nanoflagellates (Karner et al., 1994). LAPase activity has not been examined in *Prochlorococcus* because this organism has not been available in pure culture until recently, but in all likelihood it is present. As *Prochlorococcus* is of similar size (Chisholm et al., 1992) to the achlorotic *Bacteria* their respective enzyme activities in a 0.8 μm filtrate are difficult to separate. Likewise, the flow cytometric procedure used in this study did not discriminate achlorotic *Bacteria* from *Archaea*. We considered it prudent therefore, to attribute protease activities to the combined prokaryotes described. During HOT-86 neither *Prochlorococcus* nor *Synechococcus* was detected in 0.4 μm fsw, but heterotrophic bacteria in this filtrate increased rapidly in number and gave the highest per cell activity of each enzyme (with exogenous glucose plus NO_3^-) detected throughout the study. Thus, microbes in this fraction are metabolically active and capable of rapid growth.

Throughout the study, no single exogenous nutrient significantly affected LAPase activities, a finding which contrasts with that of Christian and Karl (1998) who reported that LAPase activities in water west of the Antarctic Peninsula were regulated by His. That the effects of any particular substrate on both cell production and enzyme activities varied temporally at Station ALOHA, however, may reflect changes in community structure. Heterotrophic bacterial production at this site can be regulated by substrate diversity; a combination of just two exogenous nutrients, in this case glucose and NH_4^+, was sufficient to significantly enhance numbers of heterotrophic bacteria, ectoenzyme activities and glucose uptake rates over those in unamended fsw controls. It must be said that our results do not fit a neat dichotomy of energy limitation versus nutrient limitation. They not only contrast with those of Carlson and Ducklow (1996) described above, but also with those of Pomeroy et al. (1995) who concluded bacteria in the Gulf of Mexico were phosphorus- rather than energy-limited. Rivkin and Anderson (1997), however, found results consistent with both these apparently contradictory results. The fact that during our work the effects on growth and enzyme activities of any single substrate varied widely among cruises, is a reminder that temporal aspects deserve full consideration. Conclusions based on only one cruise or a short time period are not necessarily flawed, but should be treated cautiously when extrapolated to describe an entire ecosystem.

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