Limnol. Oceanogr., 40(6), 1995, 1042–1049

Bacterial ectoenzymes in marine waters: Activity ratios and temperature responses in three oceanographic provinces

James R. Christian and David M. Karl
School of Ocean and Earth Science and Technology, University of Hawaii, Honolulu 96822

Abstract

Ectoenzymatic hydrolysis is a crucial first step in bacterial utilization of polymeric dissolved organic matter (DOM). Variation in the relative activities of different enzymes can indicate seasonal and geographic variation in the mode of bacterioplankton nutrition. We found that relative activities of leucine aminopeptidase and β-glucosidase in seawater varied significantly among three oceanic regions: the subtropical North Pacific, the equatorial Pacific, and the Southern Ocean. The temperature responses of these enzymes also vary significantly among these three regions, suggesting distinct bacterial phenotypes with distinct isozymes. Our results suggest a latitudinal trend in bacterial carbon and nitrogen utilization, with significant synthesis of cell constituents from glucose and ammonium in equatorial waters but little such de novo synthesis in Antarctic waters. The observed patterns have important implications for the parameterization of secondary production and nutrient regeneration in global production models and for understanding the role of DOM in global carbon and nitrogen fluxes.

Fluorogenic tracers of ectoenzymatic activity in marine and freshwaters have now been in use for more than a decade (Hoppe 1983; Somville and Billen 1983). Because of its utility in determining rates and patterns of bacterial utilization of dissolved organic matter (DOM) and the relative ease of measuring it, the protocol is becoming one of the cornerstone methods of aquatic microbial ecology (Hoppe 1991, 1993). Most studies of ectoenzymes to date have been conducted in freshwater, estuarine, and nearshore marine environments (Chróst 1991), i.e. in relatively eutrophic environments that usually have significant inputs of allochthonous organic matter. The overwhelming majority of such studies have been in the temperate zones. Although some data have been collected from oceanic (Hoppe 1991), polar (Billen 1991), and tropical (Hoppe et al. 1988) environments, these studies reported the activity of only a single enzyme (leucine aminopeptidase).

Most studies of bacteria in seawater have focused on estimating biomass and rates of biomass production, and information regarding the substrates utilized in situ is extremely limited. Measurements of ectoenzymatic activity can complement these other studies in several important ways. First, such measurements can be used to estimate the utilization rates of different components of DOM and thereby can also constrain estimates of biomass production and growth efficiency. Second, the enzymes that are expressed by bacteria in a given environment can give clues to the patterns of substrate utilization in that environment that are not apparent from uptake rates of macromolecular precursors such as thymidine or leucine. Finally, the temperature responses of the enzymes in a given environment can also indicate phenotypic differences among bacterial communities.

We measured the activities and temperature responses of leucine aminopeptidase (LAPase) and β-glucosidase (BGase) in three oceanic provinces from which few data regarding ectoenzymatic activities of natural bacterioplankton communities have been published: the subtropical gyre of the North Pacific, the Pacific equatorial current system, and the high latitude, permanently cold waters of the Southern Ocean. The existence of a latitudinal gradient in the relative activities of proteases and polysaccharases of marine bacteria was first observed by Kriss et al. (1963), who used less quantitative selective enrichment methods. Using 4-methylumbelliferyl-β-glucoside (MUBG) and L-leucyl-β-naphthylamine (LLBN) as tracers for BGase and LAPase in natural bacterioplankton communities, we now demonstrate that the relative activities of these enzymes show very pronounced differences among latitudinal and climatic zones and that these differences follow a latitudinal pattern that is at least qualitatively similar to that observed with the older methods.

Materials and methods

Study areas—Samples were collected from oceanic (Pacific subtropical gyre and equatorial Pacific) and polar (Antarctic Peninsula region of the Southern Ocean) en-

Acknowledgments

This work was supported in large part by NSF grants OCE 88-00329, OCE 93-01368, DPP 88-18899, and OPP 91-18439 to D. M. Karl. The equatorial Pacific cruise was supported by NSF grant OCE 90-22321 to R. R. Bidigare. James Christian was supported by a NASA Global Change Fellowship.

We thank the captains and crews of the RV *Kila,* the RV *Moana Wave,* the RV *Nathaniel B. Palmer,* the RV *Polar Duke,* the RV *Thomas G. Thompson,* and the RV *Townsend Cromwell.* We thank J. Dore, T. Houlihan, J. Kirshtein, and G. Tien for help with sample collection and preparation at sea and H. Ducklow for use of his incubators on TT-008. We thank the staff of Palmer Station and Antarctic Support Associates for technical assistance and the HOT program staff for hydrographic and sampling support. David Kirchman and three anonymous reviewers made helpful comments on earlier drafts of this paper.

SOEST contribution 3827. US-JGOFS contribution 179.

vironments. In the subtropical Pacific, experiments were conducted at the US-JGOFS time-series station ALOHA (22°45′N, 158°00′W; cf. Karl and Winn 1991); in the equatorial Pacific, they were conducted in the US-JGOFS study area at 0°, 140°W, in the South Equatorial Current (Murray et al. 1994). In the Southern Ocean, field studies were conducted in the Palmer Peninsula Long-Term Ecological Research (LTER) study area (Quetin and Ross 1992; Waters and Smith 1992). The equatorial data were collected on a single cruise in March–April 1992 (RV *Thomas G. Thompson* cruise TT-008). The other two study areas were visited repeatedly from 1991 through 1994. Antarctic data are from four cruises. RACER 3 and 4 (December–January 1991–1992 and July–August 1992) were conducted primarily in Gerlache Strait; NBP93-02 (March–May 1993) and PD94-01 (January–February 1994) covered a broader area, both inside and outside the coastal island chain along the Antarctic Peninsula. Some of the data on temperature responses for Antarctic LAPase were collected on PD92-09 (November 1992). All data presented here are from the euphotic zone (≤20 m in Gerlache Strait, ≤80 m elsewhere).

Ectoenzyme assays—The methods used here are based on those of Hoppe (1983) and Somville and Billen (1983); a useful summary is given by Hoppe (1993). The fluorescent substrate analog (MUBG or LLBN) was added to 6 ml of seawater and incubated for 12–24 h. On RACER 4 (winter cruise in Antarctica), incubation time was increased to 48 h. The sample was then poisoned with 0.1 ml of a saturated solution of mercuric chloride (final concn, ~4 mM) to stop the reaction and frozen until analyzed (cf. Christian and Karl 1995). Mercuric chloride precipitates excess LLBN which was removed by filtration (0.2 μm) or centrifugation prior to fluorescence determination. All incubations were conducted in the dark at approximately in situ temperature. Incubation temperature was 28°C at the equator and 0°C in Antarctica. At station ALOHA, incubation temperature varied slightly on different cruises, and the data presented here are normalized to 25°C.

All of our routine determinations were conducted at saturating substrate concentration (1.6 μM MUBG, 1 mM LLBN) to facilitate comparison of total enzyme among samples; they should therefore be considered potential activities rather than estimates of activity in situ. This potential activity is described as $V_{sat} = k_2 E_o \sim V_{max}$, where k_2 is the rate constant for the catalytic (hydrolysis) step, and E_o is the total enzyme present in the sample.

Fluorescence was measured in a Perkin-Elmer LS-5 or LS-5B spectrofluorometer. External fluorescence standards were prepared according to Christian and Karl (1995). Fluorescence was corrected for abiotic hydrolysis and background fluorescence of the substrate analog by subtracting the fluorescence of control samples with mercuric chloride added at time zero (Christian and Karl 1995). In some cases fluorescence of BGase samples was less than or equal to the control value, so that activity was considered to be zero. V_{sat} (in nmol liter^{-1} d^{-1}) was calculated as $(F - F_0)\, A\, (24/t)$, where F_0 is the control fluorescence, A is the concentration in nM given by 1 fluorescence unit, and t is the incubation time in hours.

Fluorescence of 4-methylumbelliferone (4MUF) was measured at 447 nm with excitation at ~360 nm. Because the pH of seawater is lower in Antarctica than at our other two study sites, the excitation spectrum of 4MUF is shifted toward shorter wavelengths, and its overlap of the excitation spectrum of MUBG is increased. Background fluorescence is decreased and the sensitivity of the method improved if the fluorescence is determined at an excitation wavelength greater than the maximum for free 4MUF fluorescence. Excitation and emission wavelengths for β-naphthylamine (BNAPH) were 337 and 411 nm, respectively. Bandwidths were 10 nm in all cases.

BNAPH experiences some loss of fluorescence with long-term storage (Christian and Karl 1995). Because our samples were stored for varying lengths of time, there is some additional variance associated with this effect. Because of uncertainty about the exact rate of loss of fluorescence, we have not attempted to correct these values to their presumed initial concentration. BNAPH activities from RACER 3 and 4 are probably underestimated by 10–20%, and in the equatorial Pacific by ~30%.

For simplicity, the enyzmes hydrolyzing MUBG and LLBN are referred to as β-glucosidase (EC 3.2.1.21) and leucine aminopeptidase (EC 3.4.1.1) respectively. However, it should not be assumed that the enzymes of natural seawater bacteria are identical to those described in the historical enzymological literature (e.g. Delange and Smith 1971) or that in vivo activities can be attributed to a single enzyme. Rath and Herndl (1994) found two distinct isozymes of BGase in samples taken from macroparticulate matter in the Adriatic Sea. Although diversity was low in this microenvironment, their results suggest that seawater samples should not be considered analogous to samples of individual enzymes in vitro.

Sampling and replication—Water was collected in Niskin or GoFlo bottles, dispensed directly into polycarbonate or HDPE bottles, and subsampled by pipet into the incubation containers (generally scintillation vials for BGase and 15-ml polypropylene centrifuge tubes for LAPase). Where duplicates or triplicates were taken, the value presented is the mean of the subsamples taken from a given Niskin bottle. At station ALOHA, LAPase activities are the mean of two replicates and BGase activities are the mean of three. In the equatorial Pacific, activities are the mean of duplicate subsamples. In Antarctica, in most cases a single subsample was taken from each depth. Variance among duplicate or triplicate subsamples was comparable to or less than in the other two study sites. In the temperature response experiments, all subsamples are shown.

In the equatorial Pacific, three of 30 samples had an LAPase activity in one of two replicate subsamples much greater than in the other, and the lower value was consistent with the rest of the data. We attribute these anomalies to the presence of particles rich in microbial activity (Smith et al. 1992). Alternative estimates of the mean

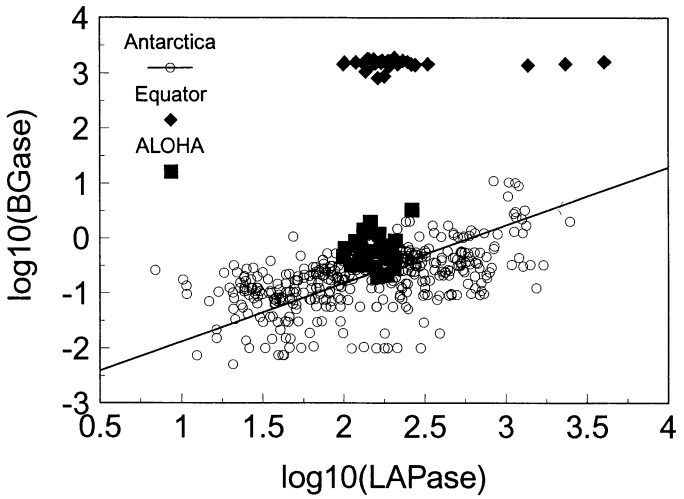

Fig. 1. LAPase vs. BGase (V_{sat}, $\log_{10}$ of rates in nmol liter^{-1} d^{-1}) at in situ temperature in the three study areas. The regression equation (model 2, geometric mean method) for the Antarctic data is $\log_{10}(\text{BGase}) = -3.01 + 1.066 \log_{10}(\text{LAPase})$ ($r^2 = 0.311$, $P < 0.001$, $n = 398$).

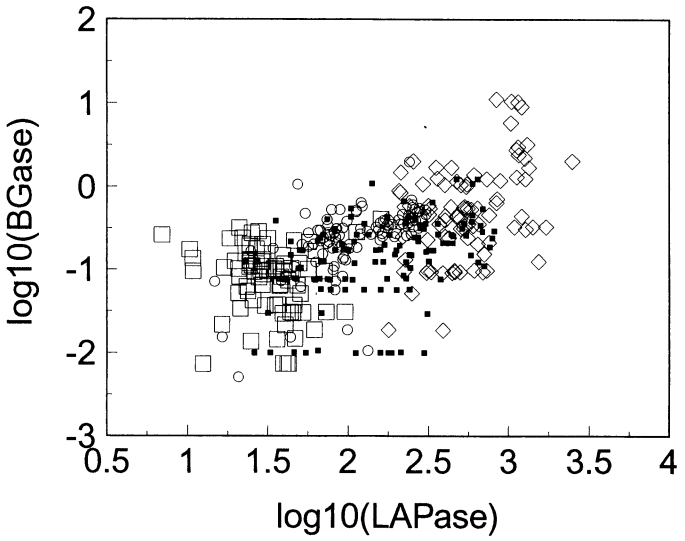

Fig. 2. LAPase vs. BGase (V_{sat}, $\log_{10}$ of rates in nmol liter^{-1} d^{-1}) at in situ temperature on four Antarctica cruises in various seasons: December 1991 (RACER 3—◇), July–August 1992 (RACER 4—□), March–May 1993 (NBP93-02—○), and January–February 1994 (PD94-01—■).

activity that do not include these three points are given in Table 1.

Temperature control—The activities of these enzymes at various temperatures were determined in several experiments. These were carried out in temperature-controlled water baths or in a set of flowthrough deck incubators designed at the University of Hawaii. To determine the response to finer gradations of temperature, we constructed a device known as the "thermatron," which consists of an aluminum block ~80×40×7 cm with 36 holes (9×4) each containing a 50-ml beaker of seawater in which samples can be placed. At either end of the block, water flows through an opening ~0.5 cm in diameter spanning the width of the block. Water was circulated through these openings from two temperature-controlled water baths whose temperatures differed by ~15°C, creating a gradient along the block of ~8°C in ~1°C intervals between samples. The block was housed in an insulated box made of 1.27-cm polyvinylchloride sheeting with styrofoam lining ~8 cm thick. When the temperature of the water bath at the cold end of the gradient was <0°C, ethylene glycol was added to prevent freezing.

Table 1. Mean values of V_{sat} (nmol liter^{-1} d^{-1}) for LAPase and BGase for the three study areas and for four Antarctic cruises. LAP : BG are ratios of the mean values for each environment or cruise.

Location	*n*	LAPase	BGase	LAP : BG
Equator	30	420	1,519	0.276
Outliers removed	30	192	1,519	0.127
ALOHA	28	160	0.75	213
Antarctica				
All cruises	398	260	0.44	593
Dec 1991	90	648	1.18	548
Jul–Aug 1992	80	37	0.11	339
Mar–May 1993	105	137	0.32	434
Jan–Feb 1994	123	228	0.22	1,052

Results

The ratio of LAPase to BGase (V_{sat}/V_{sat}) varied significantly and systematically among the different study areas. LAPase : BGase at the equator was much less than in the other two regions. BGase at the equator was 3–4 orders of magnitude greater than at station ALOHA, while LAPase was about the same (Fig. 1 and Table 1). LAPase : BGase at station ALOHA was generally less than in Antarctica (Table 1). The difference in LAPase : BGase among the three study areas was in all cases highly significant [$P < 0.001$; Wilcoxon two-sample test (nonparametric) or one-way ANOVA on log-transformed data].

The potential activity (V_{sat}) of LAPase in the subtropical Pacific and in Antarctica was 2–3 orders of magnitude greater than that of BGase. Because of seasonality, both enzymes had a much greater range in Antarctica than in the other two regions. For each of four cruises in Antarctica, there is a cluster of points with variable LAPase : BGase ratio (i.e. the points do not fall along a line), and the mean ratio varied among the four cruises (Fig. 2 and Table 1). Overall, there was a strong positive correlation ($P < 0.001$) but a large amount of variance ($r^2 = 0.311$) These data span a large trophic gradient, and correlations within a given area and season can be weak (Christian and Karl 1992). The regression equation is given in the legend to Fig. 1.

The results from four cruises in Antarctic waters suggest

Table 2. Regression statistics for temperature dependence of ectoenzyme activity [model 1 linear regression of $\log_{10}(V_{sat})$ on incubation temperature in °C].

Enzyme	Location	Intercept	Slope	r^2
LAPase	Antarctica	0.93	0.023	0.49
LAPase	Station ALOHA	−0.18	0.042	0.97
LAPase	Equator	−2.76	0.124	0.98
BGase	Antarctica	−1.85	0.022	0.98
BGase	Station ALOHA	−0.07	0.041	0.96
BGase	Equator	−5.12	0.234	0.96

seasonal variation in the substrates utilized by bacterioplankton. The LAPase : BGase ratio was greatest in summer and least in fall and winter, implying that glucose is more heavily utilized as the supply of dissolved proteins and peptides produced by spring–summer bloom processes is exhausted. LAPase : BGase on each of the summer cruises was significantly ($P < 0.001$, Wilcoxon or ANOVA as above) different from the fall and winter cruises; differences between the two summer cruises and between the fall and winter cruises were not significant at $\alpha = 0.05$. The turnover rate of this substrate pool would therefore be on the order of several months but, since activities decline quite precipitously during winter, <1 yr. This effect is more apparent in the more oligotrophic shelf waters surveyed during January–February 1994 (PD94-01) and March–May 1993 (NBP93-02) than in the RACER study area in Gerlache Strait. In the open shelf waters, LAPase activities were as great in autumn as in summer, while BGase activities were higher in autumn (Fig. 2 and Table 1). LAPase activities on the autumn cruise, however, declined rapidly as winter approached (Christian and Karl 1993).

The temperature responses of these enzymes in the three regions differed significantly, which may reflect important differences among bacterial communities (Fig. 3). The temperature relationships at station ALOHA were more similar to those in samples from Antarctica than to equatorial waters, although the surface-water temperature at station ALOHA (23–27°C) was very similar to that in the equatorial study area, which is within the same range at most times. More extreme temperatures are observed in association with El Niño events, and temperatures during March–April 1992 were among the warmest recorded in 12 yr of continuous measurement (TOGA-TAO array). Water temperature in Antarctica is near 0°C at all times of the year. All regression lines have $r^2 > 0.95$ except for Antarctic LAPase ($r^2 = 0.49$), which is a composite of several experiments with similar slopes but different intercepts (Table 2). The slopes of the temperature relationships at station ALOHA are greater than those in Antarctica by a factor of ~2 (Table 2). Both enzymes seem to show a reduced response to temperature as an adaptation to colder water. The coincidence of the slopes of the activities for these two enzymes is quite remarkable. It is also interesting that the slopes are so much greater in the equatorial Pacific than at station ALOHA, given the small difference in water temperature.

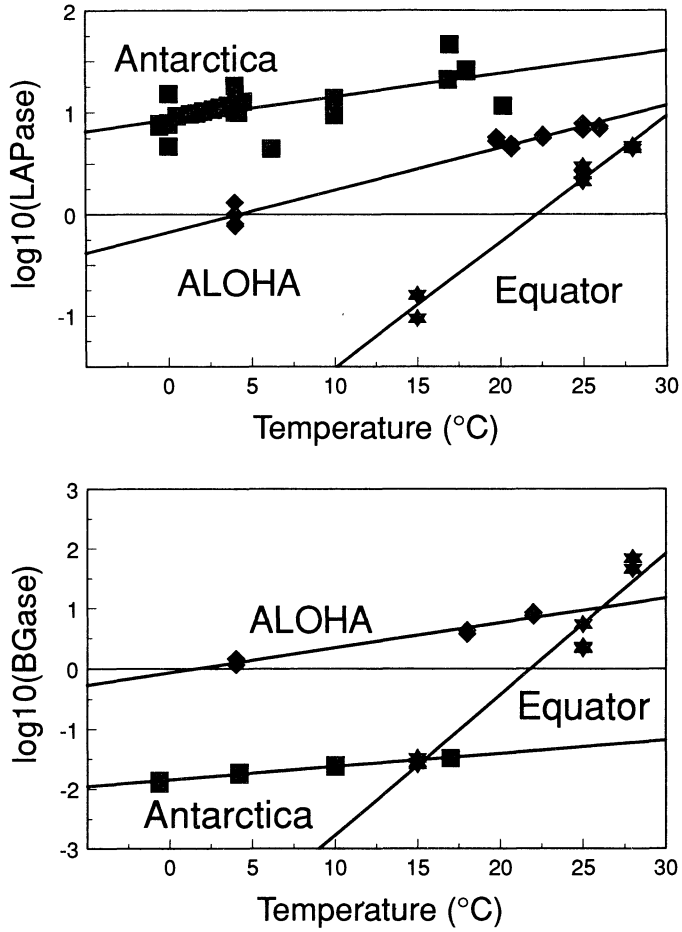

Fig. 3. Temperature dependence of V_{sat} for LAPase (top) and BGase (bottom) ($\log_{10}$ of rates in nmol liter^{-1} d^{-1}). Activities at station ALOHA are concentrated by a factor of 33 (intercept is offset by 1.52). Regression statistics are given in Table 2.

V_{sat} for LAPase in Antarctica did not have a temperature optimum over the range 0–20°C (maximum summer water temperature is 1–2°C). At low substrate concentrations (nearer to those actually present in the ocean), the reaction was only weakly dependent on temperature (Fig. 4). This apparent temperature independence may result because the rate-limiting step in this concentration range (substrate binding) is reversible. Because the rate constants for both forward and back reactions presumably increase with temperature, there is no increase in rate of the overall reaction. Only when substrate concentration increases and the second, nonreversible step (hydrolysis) becomes rate limiting does temperature become a significant consideration.

The extremely oligotrophic subtropical gyres may represent something approaching global minima in both activities. Moving away from this minimum, it appears generally true that LAPase increases toward the poles and BGase toward the equator. However, this conceptual model must be qualified by emphasizing that our data come only from the extremes of latitude, and these trends

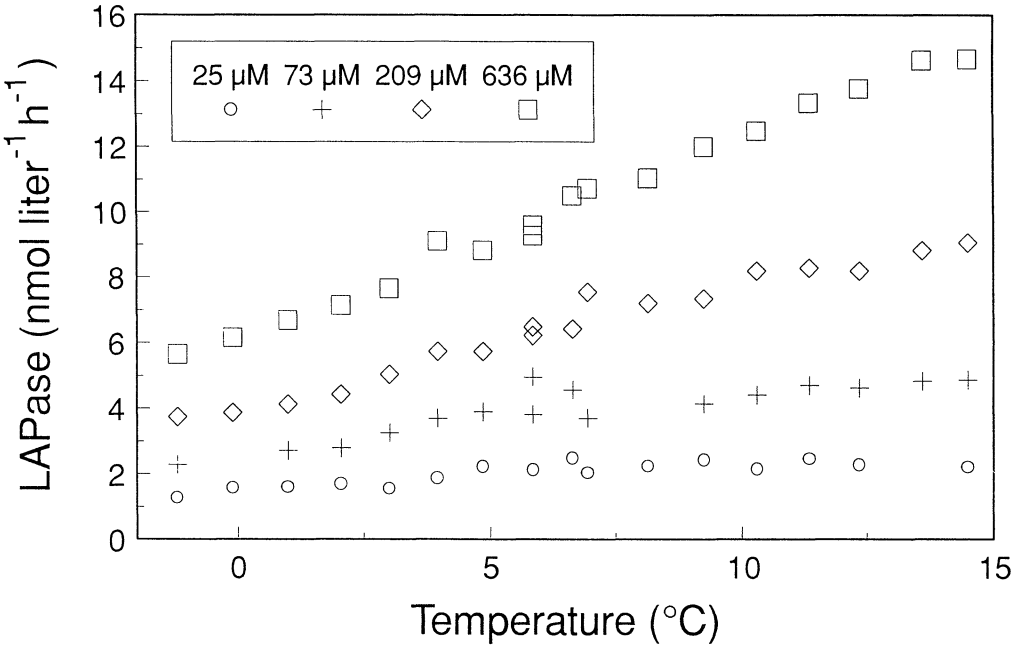

Fig. 4. LAPase activity in Antarctic seawater at incrementally increasing temperature in the "thermatron" at four concentrations. Composite of two experiments normalized to 636 µM rate in region of overlap.

may not hold in the midlatitudes. In mid- and high-latitude environments, moreover, these trends are complicated by the effects of seasonality. If our data have elucidated the true global minima for the activities of these enzymes in seawater, they are to be found in the Antarctic winter rather than in the subtropical gyre (Table 1). Both BGase and LAPase in Antarctica in summer can be as great as or greater than at station ALOHA; however, the ratio of LAPase to BGase is generally greater in Antarctica.

Discussion

The latitudinal pattern of activity ratios described here bears an interesting resemblance to patterns observed by Kriss et al. (1963), who isolated more than 4,000 bacterial strains on extensive cruises in all of the major ocean basins (Fig. 5). Qualitatively, our results show a similar trend with latitude, with glucosidase activity predominating in the equatorial zone and proteases increasing in importance with increasing latitude. However, the data of Kriss et al. (1963) depict a more or less continuous latitudinal gradient in activity ratios, whereas our results show a relatively small difference in the LAPase : BGase ratio between our subtropical and polar study areas and an altogether unique situation in the equatorial zone.

Although there are few data from oceanic waters, LAPase activities in the midlatitudes of the Northern Hemisphere seem to be comparable to our Antarctic data (Hoppe 1991), which might be interpreted as meaning that bacteria in temperate to polar waters subsist largely on peptides and amino acids. However, the affinities of oceanic LAPase and BGase for their respective substrates are quite different. LAPase has a much higher k_m (Somville and Billen 1983; Somville 1984; unpubl. data), suggesting that LAPase activity in situ is significantly less than V_{sat}, while BGase may be active at or near V_{sat} much of the time.

Recent data suggest that dissolved polysaccharide concentrations in the ocean are much greater than previously believed (Benner et al. 1992; Pakulski and Benner 1994). However, neutral sugars may constitute only a small fraction of this pool, and the enzymes hydrolyzing 4MUF-glucosides do not necessarily hydrolyze the bonds present in naturally occurring polysaccharides, especially those involving acidic and amino sugars. Whatever the abundance of glucose or other neutral sugars in the dissolved polysaccharide pool, a low level of constitutive BGase activity may be sufficient to supply the bacteria with all the glucose they can utilize given the mixture of other substrates available and the biosynthetic pathways that are active. For example, while bacteria may be genetically capable of synthesizing amino acids from glucose and ammonium, this pathway is not necessarily active, and in practice, most cell protein may be synthesized from amino acid precursors. Respiration of significant amounts of amino acid carbon by Antarctic bacteria (Tupas et al. 1994) suggests that this is the case.

Wheeler and Kirchman (1986) found that bacterial growth in Gulf Stream water was stimulated by glucose and ammonium to a greater extent than by stoichiometrically equivalent (with respect to C and N) amounts of an algal amino acid mixture. Kirchman (1990) found the opposite to be true in the subarctic Pacific, where glucose

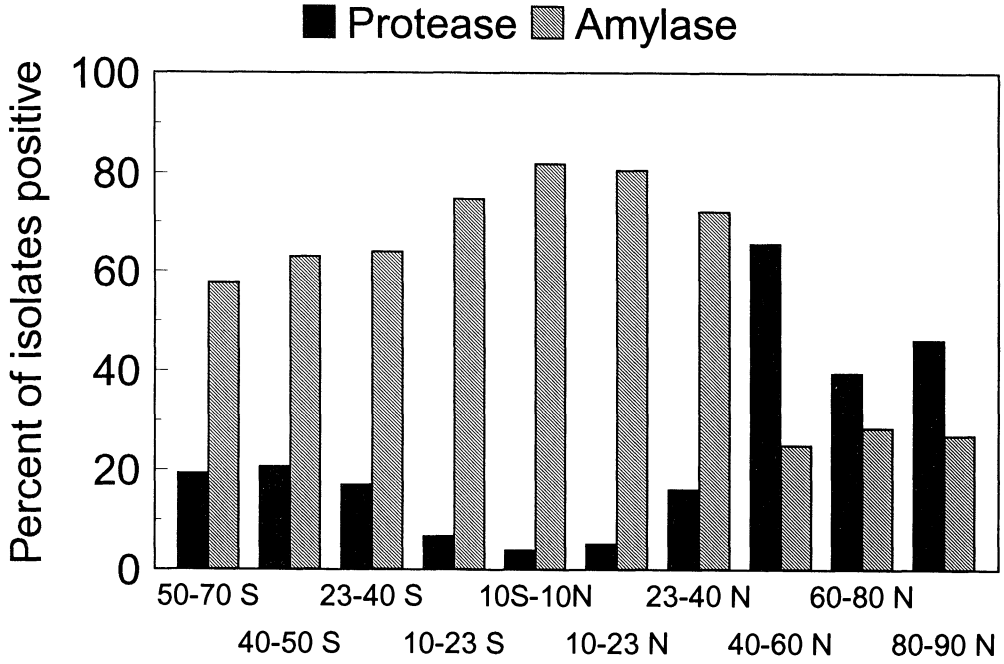

Fig. 5. Percent of bacterial strains isolated from seawater hydrolyzing starch (amylase) and gelatin (protease) (from Kriss et al. 1963).

and ammonium stimulated growth but to a lesser extent than amino acids did. These results are consistent with our hypothesis of a latitudinal trend in bacterial utilization of sugars and polysaccharides relative to amino acids and proteins. The subarctic Pacific shares certain oceanographic characteristics with waters of the Southern Ocean (e.g. low temperatures, salinity stratification, permanently high inorganic nutrient concentrations). Gulf Stream water is largely derived from the Sargasso Sea and other tropical and subtropical seas (Gulf of Mexico, Caribbean Sea) with some indirect input from the South Equatorial Current (Froelich et al. 1978; Pickard and Emery 1982).

The studies cited above offer some evidence in support of the biogeographic patterns hypothesized in this paper, although the actual patterns are likely to be complex, and much remains to be learned about the rates of acquisition of various substrates by marine bacteria and their fates once assimilated. Bacterial uptake of ^{3}H- or ^{14}C-labeled glucose is easily demonstrated, but quantitative information on partitioning between respiration and synthesis of carbohydrate and noncarbohydrate cell constituents is scarce for oceanic systems. Similarly, amino acids may be respired or used in protein synthesis, and the percent respired varies significantly among the few amino acids that have been studied (Tupas et al. 1994). For most amino acids, particularly those not abundant in seawater, the partitioning between assimilation and respiration and between de novo synthesis and synthesis from intermediate precursors available in seawater is largely unknown for native aquatic bacteria. It is reasonable to assume that many, if not most, natural bacterioplankton are auxotrophic for at least some substrates, as are many bacteria in culture (Guirard and Snell 1962).

The different temperature responses at each study site indicate that distinct bacterioplankton populations with different enzymes or isozymes have evolved in each region. Antarctic populations do not display a temperature optimum for k_2 near in situ temperatures, and weak temperature dependence at low substrate concentration suggests that a k_2 optimized at low temperatures offers little selective advantage. However, enzymes from Antarctic microenvironments, where substrate concentrations may be much higher (sediments, sea ice, decaying macrophytes), also do not display temperature optima approaching in situ temperature (Reichardt and Dieckmann 1985; Helmke and Weyland 1991) so this may also reflect fundamental physicochemical constraints that natural selection cannot overcome. The slope of the temperature response can also be interpreted as an adaptation to low temperatures (i.e. a lowered activation energy barrier), which is generally observed in cold-adapted enzymes (Low et al. 1973; Somero 1978).

Activities from the different regions can be normalized to a constant temperature, but because the slopes of the temperature-activity relationships are different for the three environments, the activity ratio depends on the temperature chosen. Normalized temperatures cannot be interpreted in terms of relative E_o because the substrate affinities of the different isozymes are likely to be different at any given temperature. However, it should be noted that although the surface water temperature in the equatorial Pacific during March–April 1992 was high, the BGase activities observed would still be much greater than those at station ALOHA if they were normalized to a temperature nearer the climatological mean.

The high k_m for LAPase in planktonic bacteria is in-

triguing given the low concentrations of dissolved peptides and proteins in seawater (<1 μM, e.g. Druffel et al. 1992). Maintaining high constitutive levels of an enzyme that consistently operates at only a small fraction of its capacity does not seem energetically optimal. Either there is something inherit in the enzyme that prevents higher affinity isozymes from evolving, or, for reasons unrelated to energetics, there is little selective advantage to such an adaptation. The apparent half-saturation constants for solubilization of dissolved proteins are much lower (Hollibaugh and Azam 1983), suggesting that liberation of individual amino acid residues by terminal aminopeptidases (exohydrolases) may be the rate-limiting step in bacterial utilization of concentrated patches of proteinaceous matter, implying a flux of peptides of intermediate size into the surrounding medium (Azam and Cho 1987). It is interesting that many free-living marine bacteria seem to have evolved along an opposite course with respect to the glucosidases: high substrate affinity and synthesis or induction only in response to certain environmental conditions. BGases with much higher k_m have been reported for eutrophic lakes (Chróst 1989), although high-affinity isozymes have also been reported from eutrophic environments (Somville 1984).

Very little is known about the evolution and taxonomy of free-living oceanic bacterioplankton. The bacterial community in the equatorial Pacific seems to be physiologically quite distinct. Whether these bacteria are taxonomically distinct is unknown, and it is possible that these physiological adaptations occur within the taxonomic groups delineated by molecular methods such as ribosomal RNA analysis (Ward et al. 1992) and are not presently detectable using such methods. It is also possible that the extremely temperature-sensitive isozymes expressed by equatorial bacterioplankton during March–April 1992, within the warm-water pool of El Niño, are not present at other times. Although it appears that these enzymes are ideally adapted to rapid "shift up" in a region of variable temperature, it is also possible that these bacteria are the culmination of a succession process that followed a change in water temperature and are not representative of the community in this region at other times. BGase activities in August 1992, when the study site was no longer within the warm-water pool (Murray et al. 1994), remained orders of magnitude above those at our other two study sites (unpubl. data), but temperature experiments were not conducted.

The extremely high levels of BGase activity near the equator have important implications for the role of DOM in the global carbon cycle. Initial reports of a low C:N for persulfate-resistant DOM appear to be incorrect (Hedges and Lee 1993), and oceanic DOM contains a large amount of polysaccharide (Benner et al. 1992; Pakulski and Benner 1994). If, for example, 10 μM dissolved organic C is hydrolyzable by the enzymes that hydrolyze MUBG, this fraction will have a turnover time of 1–2 d at the equator and ~6 yr in the subtropical North Pacific. Little is known about the composition of oceanic DOM and the biological turnover rates of the various fractions. If the turnover rates of significant fractions vary by several orders of magnitude, simulations of the global carbon cycle incorporating DOM (e.g. Najjar et al. 1992) will be made much more realistic if the rates of these processes are better constrained.

Marine microbial ecology remains a methods-limited science in many respects. There has been a tendency to focus on biomass and biomass production; there is much less information on the kinds of substrates that sustain natural populations in the ocean. Our inability to characterize much of the DOM in the ocean has encouraged a "stoichiometric" as opposed to a "biochemical" approach. Organisms are viewed in terms of C, N, and P rather than protein, carbohydrate, etc., although different compounds containing similar amounts of each element are unlikely to be equivalent to the organism. The biochemical compounds utilized by the bacterioplankton may differ substantially among ocean basins and regions, and identifying oceanographic provinces within which consistent patterns of substrate utilization occur is an important step toward incorporating the bacteria into ecological models and defining appropriate experimental protocols for routine use in the field.

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Submitted: 29 September 1994
Accepted: 1 March 1995
Amended: 1 May 1995