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Patterns of extracellular enzyme activities among pelagic marine microbial communities: implications for cycling of dissolved organic carbon

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ABSTRACT: The potential for pelagic microbial communities to hydrolyze high molecular weight substrates and therefore to initiate remineralization of high-molecular-weight dissolved organic carbon (DOC) was assessed at 8 different stations spanning a range of locations. Six structurally diverse polysaccharides were used to investigate activities and structural specificities of a related class of microbial extracellular enzymes, the polysaccharide hydrolases. Three high-latitude (57 to 79° N) stations showed similar rates and patterns of enzyme activity, with only 3 of 6 polysaccharides hydrolyzed. Hydrolysis at the other stations (39° S to 29° N) showed a variety of patterns, in which 2 to 5 of the polysaccharides were hydrolyzed. One of the polysaccharides, fucoidan, was not hydrolyzed at any station, while only laminarin was hydrolyzed at every station. A limited ability of microbial communities in some locations to hydrolyze high-molecular-weight substrates could help explain the persistence of a fraction of DOC in the ocean. Potential hydrolysis rates showed no correlation with factors such as environmental temperature or total cell numbers. Denaturing gradient gel electrophoresis analysis of community 16S rDNA indicated that the microbial communities at these locations were diverse, consistent with the diversity in patterns of enzyme activities.

KEY WORDS: Extracellular enzyme · DOC · Carbon cycling · Polysaccharide

INTRODUCTION

Microbial communities in pelagic systems play a key role in the cycling of organic carbon and nutrients. An estimated 50% of primary productivity is recycled via the microbial loop (Azam 1998). Much of the organic carbon produced and excreted by phytoplankton is in the form of chemically complex macromolecules. Since prokaryotes with an outer cell membrane can only transport low-molecular-weight (<600 Da; Weiss et al. 1991) substrates across this membrane, larger substrates must initially be hydrolyzed by extracellular enzymes. Such enzymes are selective with respect to substrates, since they can hydrolyze only structures that correspond to the enzymes' active sites (Warren 1996). The structural specificities and activities of microbial extracellular enzymes are, therefore, key factors affecting the cycling of high-molecular-weight organic carbon in marine systems

The range of tools that has been applied to investigate the activities and structural specificities of extracellular enzymes is somewhat limited. Most measurements of enzyme activities in aquatic environments have been made using small substrate proxies, consisting of a monomer such as glucose or leucine linked to a fluorophore whose fluorescence increases upon hydrolysis (e.g. Christian & Karl 1995, Martinez et al. 1996, Fukuda et al. 2000). While these small substrate proxies

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are easy to apply, widely used, and facilitate intercomparison of data from different studies, they lack the structural complexity of true macromolecules and cannot effectively mimic the 3-dimensional structure of complex macromolecules in solution (Warren 1996). Alternative approaches, more suitable for investigation of enzymatic specificity, have also been developed. These methods also use fluorescence detection, but the substrates whose hydrolysis is investigated are oligo- and polymers (Arnosti 1995, Pantoja et al. 1997). With such substrates, structurally based distinctions in hydrolysis rates and patterns of macromolecules have been investigated in a number of studies (e.g. Arnosti 1998, 2000, Arnosti & Holmer 1999, Pantoja & Lee 1999).

The cycling of carbohydrates is particularly relevant to investigations of microbial metabolism, since carbohydrates are major constituents of phytoplankton (Parsons et al. 1961), marine particles, and sediments (Cowie & Hedges 1984). Although carbohydrates are frequently considered to be labile substrates for microbes, the high contribution of carbohydrates to dissolved organic carbon (DOC) in seawater (Benner et al. 1992) as well as sedimentary pore waters (Arnosti & Holmer 1999) demonstrates that not all carbohydrates are easily metabolized. What factors distinguish labile from recalcitrant carbohydrates? Efforts to investigate this question are complicated by 2 factors: (1) the monosaccharides that make up complex carbohydrates can be linked in a multitude of structures (2 hexoses can form 16 different disaccharides; 3 hexoses could form 384 different trisaccharides, for example); and (2) our ability to analyze this structural complexity in marine samples is extremely restricted. We therefore know as little about the macromolecular structure of marine carbohydrates as we do about the enzymes which microorganisms use to hydrolyze them to tractable sizes.

Given the difficulty of direct structural analysis of marine polysaccharides, another means of assessing microbial ability to utilize complex carbohydrates in marine systems is to measure hydrolysis rates of well-characterized complex polysaccharides. This is the approach that we took in a previous study in which the hydrolysis rates and patterns of 6 structurally distinct polysaccharides (pullulan, laminarin, xylan, fucoidan, arabinogalactan, and chondroitin sulfate) were measured in sediments and bottom water from the Skagerrak, the transition between the North and Baltic Seas (Arnosti 2000). These polysaccharides were selected because all come from marine sources, and/or their component monomers are common in marine systems. Laminarin, xylan, and fucoidan occur in marine algae (Painter 1983); laminarinase, pullulanase, and xylanase enzyme activities have been detected in marine bacteria (Wainwright 1981, Arnosti & Repeta 1994, Araki et al. 2000); chondroitin sulfate is produced commercially (Fluka) from shark cartilage, and arabinose and galactose are among the monosaccharides most commonly detected in particles, sediments, seawater, and plankton (e.g. Cowie & Hedges 1984, Biersmith & Benner 1998). The 6 polysaccharides were hydrolyzed in Skagerrak sediments at different rates, but more surprising was the fact that 3 of these 6 polysaccharides — pullulan, fucoidan, and arabinogalactan — were not hydrolyzed in the overlying bottom water. An investigation of a river-bay-shelf transect during 3 seasons has additionally shown a lack of measurable hydrolysis of some polysaccharides in the water column (Keith & Arnosti 2001). How widespread are such patterns? Could these types of polysaccharides contribute to the persistent carbohydrate fraction of DOC?

In this study, we took advantage of cruises of opportunity to extend our previous investigations of extracellular enzymatic hydrolysis to a wider geographic range of water column stations, in an effort to investigate patterns of extracellular enzymatic activity. At a number of the stations, we also were able to obtain additional data relevant to bulk microbial activity and surveyed microbial community composition. This investigation is the first to compare the activities of a diverse range of extracellular enzymes — all belonging to a major class of polysaccharide hydrolases — at a broad geographic range of stations. Due to inherent structural limitations, such comparative studies of diverse enzymes belonging to a single major class cannot be carried out with small substrate proxies. The current suite of 6 substrates itself represents a very small fraction of the natural diversity of polysaccharide structures. This investigation doubtless does not reflect the full extent and range of polysaccharide hydrolysis, nor does it attempt to assess the hydrolysis of other common biopolymers. Nonetheless, by including a variety of homogeneous and heterogeneous neutral and charged polysaccharides in this study, a first step has been taken towards assessing the functional diversity of microbial extracellular enzymes in marine waters.

MATERIALS AND METHODS

Sites and sample collection. The study sites ranged from 79° N in the Arctic Ocean to 39° S off the west coast of South America (Fig. 1). Station locations, sample collection depths, and temperatures are shown in Table 1. Samples were collected in April and July 1999 (Stns S4 and J, respectively), June 2000 (Stn AB), and July–August 2000 (all other stations.) Water was collected with a clean bucket (Stns AB and J) or a Niskin bottle (all other stations), and stored at *in situ* temperature in an acid cleaned polycarbonate bottle for up to

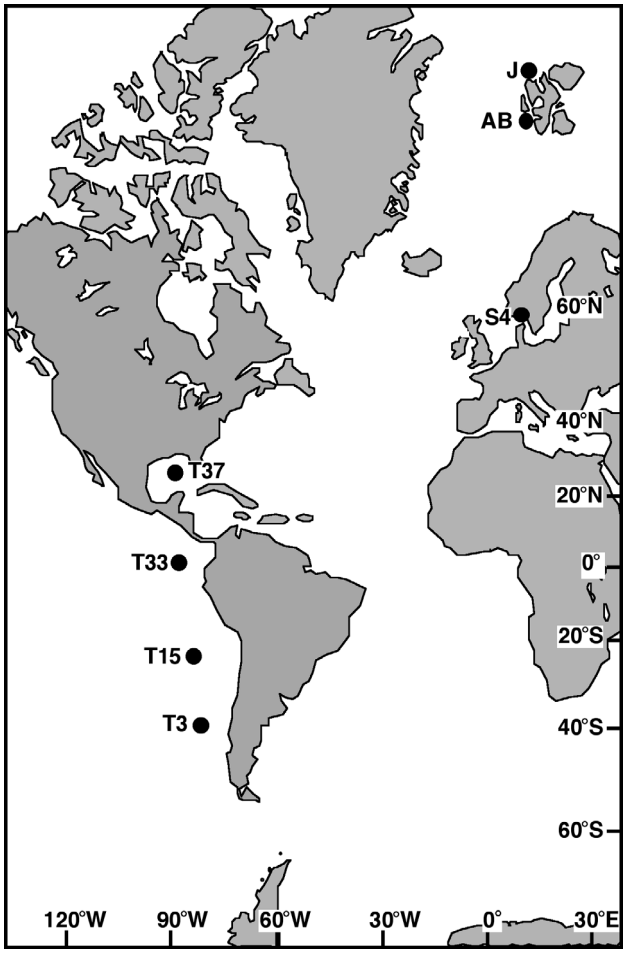

Fig. 1. Station locations

24 h before beginning the incubations. Chlorophyll *a* samples were collected on GF/F filters (Whatman), extracted on 90% acetone, and quantified fluorometrically (Parsons et al. 1984). Temperature of the samples from the Pacific Ocean and Gulf of Mexico were determined by CTD (Seabird).

Measurement of extracellular enzyme hydrolysis rates. Rates of extracellular enzymatic hydrolysis were measured using a suite of 6 structurally distinct polysaccharides: pullulan, an α(1,6)-linked polymer of maltotriose; laminarin, β(1,3)glucose; xylan, β(1,4) xylose; fucoidan, a sulfated fucose polysaccharide; arabinogalactan, a mixed polymer of galactose and arabinose; and chondroitin sulfate, a sulfated polymer of N-acetyl galactoseamine and glucuronic acid. The polysaccharides were all obtained from Sigma or Fluka, and were labeled with fluoresceinamine (isomer II; Sigma) using the procedure of Glabe et al. (1983), as modified by Arnosti (1995).

To begin each set of hydrolysis experiments, a substrate quantity equivalent to 175 nmol monomer was added to 50 ml unfiltered seawater, which was then divided into 3 replicate vials. All incubations were carried out at *in situ* temperatures (Table 1). Equivalent monomer concentrations of each substrate were added to the vials in order to keep carbon addition levels consistent among experiments, as well as to provide a measure of hydrolysis that is independent of the rate calculation. As discussed in Arnosti (2000), to a first approximation, under these conditions 2 different enzymes operating at the same rate would hydrolyze their respective substrates completely to monomers over the

Table 1. Station information. Leu incorp: leucine incorporation; –: not measured

Stn	Location	Sample depth/ water column depth (m)	*T* (°C)	Cells ml^{-1} ($\times 10^5$)	Leu incorp (pmol l^{-1} h^{-1})	Leu incorp (amol $cell^{-1}$ h^{-1})	Chl *a* (μg l^{-1})
J	79° 42.81′ N 44° 05.20′ E	1/218	4	–	–	–	–
AB	77° 35.13′ N 15° 05.70′ E	1/100	4	–	–	–	–
S4	57° 59.00′ N 09° 38.70′ E	175/190	5	–	–	–	–
T37	24° 54.61′ N 87° 45.68′ W	3/1905	30	4.10	85.8	2.09×10^{-4}	0.09
T33	01° 07.57′ N 83° 52.55′ W	3/3535	26	4.93	117	2.36×10^{-4}	0.47
T15s	23° 09.85′ S 79° 17.67′ W	3/4468	17.5	1.76	22.2–29.1[a]	1.26–1.65[a] $\times 10^{-5}$	0.16
T15d	23° 09.85′ S 79° 17.67′ W	100/4468	17.5	1.25	–	–	–
T3	39° 20.35′ S 77° 57.62′ W	3/4532	11	5.07	12.1–37.8	2.42–7.45[a] $\times 10^{-5}$	0.70

[a]Estimated between neighboring stations

same period of time, since complete hydrolysis of each polymer would yield molar equivalent monomer concentrations. A visual comparison of the relative proportions of substrates eluting in the monomer fraction from the chromatography system therefore provides an unambiguous measure of the relative order of hydrolysis rates among the 6 substrates at any given time point.

The fluorescently labeled polysaccharides and their hydrolysis products were separated chromatographically using a gel permeation chromatography system connected to a fluorescence detector, as described in Arnosti (2000). Hydrolysis rates, calculated as previously reported (Arnosti 1995, 2000), are based on integration of these chromatograms. In a number of cases, small variations in the baseline noise level account for a non-zero calculated rate, but in these cases, the error bars among the triplicates were large and the overall hydrolysis rates were not distinguishable from zero. The plotted data show only the hydrolysis rates corresponding to detectable changes in the chromatograms, in which the calculated hydrolysis rate (including standard deviation among the triplicate incubations) is distinguishably different from zero. As discussed in Arnosti (1995), all hydrolysis rates reported here are 'potential' rates.

Microbial parameters. Bacterial abundance was measured by epifluorescence microscopy of formalin preserved (2% final concentration, stored at 4°C) DAPI-stained samples (Porter & Feig 1980).

Bacterial production was estimated from measures of ^{3}H-leucine incorporation. Ten ml volumes with 10 nM ^{3}H-leucine final concentration were incubated at *in situ* temperatures for 4 h in Whirlpak bags wrapped in aluminum foil to exclude light effects (Aas et al. 1996). Triplicate 1.5 ml samples were removed from each bag at the end of the incubation, and rates of ^{3}H-leucine incorporation were determined by the microcentrifuge method of Smith & Azam (1992).

Denaturing gradient gel electrophoresis (DGGE). One liter water samples were collected onto 0.2 mm pore-size SUPOR filters (Pall Gelman) and frozen at –80°C until subsequent analysis. DNA was extracted using a boiling SDS procedure (Jeffrey et al. 1994), extracted once with phenol:chloroform and again with chloroform, and the DNA in the aqueous phase was concentrated using Centricon 100 (Amicon) cartridges (Murray et al. 1998). A 193 bp fragment of eubacterial 16S rDNA genes was amplified using GM5F-GC clamp: (CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG) and UNIV 517R (ATT ACC GCG GCT GCT GG). DGGE was performed on PCR products as described by Murray et al. (1998) and Muyzer (1999). The similarity dendrogram of DGGE banding patterns was created using Unweighted Pair Group Method with Arithmatic Mean analysis (UPGMA) with the GelCompar II Complete Software package from BioSystematica. This uses a sequential clustering algorithm in which local topological relationships are identified in order of similarity and a phylogenetic tree is built in a stepwise manner. A set of DGGE marker standards were created using DNA from an *Escherichia coli* Strain B, *Clostridium perfringens* (Cl. Welchii), *Micrococcus luteus* (*M. lysodeikticus*) (all available from Sigma Chemicals) and *Clostridium clostridiforms* (courtesy of M. Shields, Univ. West Florida).

RESULTS

Enzymatic hydrolysis patterns and rates

Two common factors characterized enzymatic activities at every site: laminarin was hydrolyzed everywhere, while fucoidan was not hydrolyzed at any station, irrespective of water temperature or incubation time (Figs. 2 & 3). The general patterns ultimately observed at the 3 northernmost stations (J, AB, and S4) were the same. Xylan, laminarin, and chondroitin sulfate were hydrolyzed, while pullulan, arabinogalactan, and fucoidan were not (Fig. 2). Hydrolysis rates of the 3 polysaccharides at each station were within a factor of 2 of one another, and the hydrolysis rate of each polysaccharide was also within a factor of 2 among the 3 stations. The more southerly stations exhibited distinctly different patterns (Fig. 3). At Stns T37 and T33, a broad range of activities was detected, with 5 of the 6 substrates hydrolyzed. Hydrolysis rates of the 5 polysaccharides were within a factor of 2 to 3 of one another at Stn T37, but spanned a greater range at Stn T33. (Note that the first sampling point at Stn T37 was 15 d. As discussed below, laminarin hydrolysis rates may have been more rapid at earlier time points.) Four polysaccharides were hydrolyzed at Stn T15s, at rates differing by as much as a factor of 4, while only 2 substrates were hydrolyzed at Stn T15d, the same location, but at a depth of 100 m. Hydrolysis rates of laminarin and arabinogalactan at Stn T15d, however, were similar to the rates measured in surface waters. At Stn T3, only 2 of the 6 substrates were hydrolyzed, laminarin at rates somewhat lower than at Stns T15d and T15s, and chondroitin sulfate at rates higher than at those same stations.

The measurement of substrate hydrolysis at several time points at each station revealed differences between substrates and among stations in the time course of development of enzyme activities. For some stations and substrates, the onset of hydrolysis was rapid and rates of hydrolysis were quite high, while in

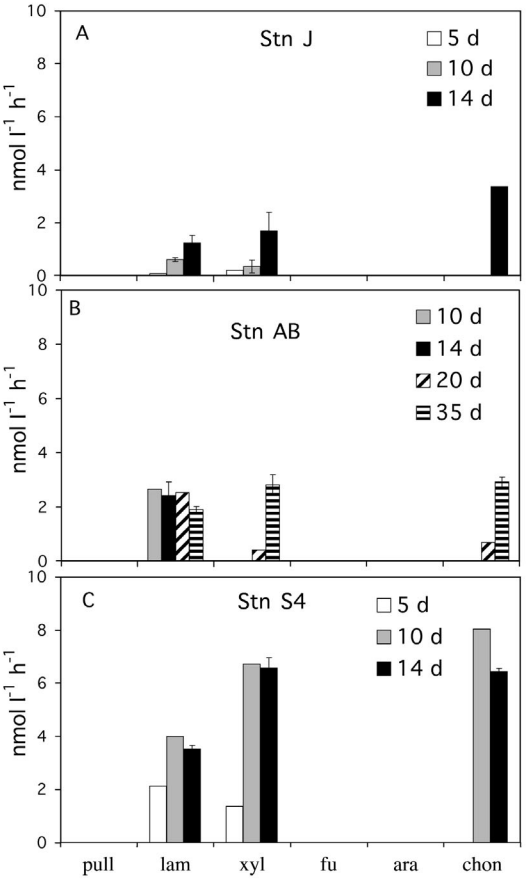

Fig. 2. Hydrolysis rates at (A) Stn J (79° N), (B) Stn AB (77° N), and (C) Stn S4 (57° N); rates from Stn S4 (C) are corrected for a spreadsheet error in Arnosti (2000). For precise station locations, see Table 1. Incubation time (d) at which hydrolysis rates were measured is shown; error bars show SD of triplicate incubations. Pull: pullulan, lam: laminarin, xyl: xylan, fu: fucoidan, ara: arabinogalactan, chon: chondroitin sulfate

other cases, there was a significant time lag before enzyme activity was detectable (Figs. 2 & 3).

Overall, the hydrolysis rates of the substrates ranged from undetectable (e.g. fucoidan at every station; all substrates except laminarin for at least 1 station) to 17 ± 4.4 nmol l^{-1} h^{-1} for xylan at Stn T33. At any given station, hydrolysis rates differed by factors of ca. 2 to 7 (Figs. 2 & 3). Pullulan hydrolysis rates ranged from undetectable at the northern stations to 3.4 ± 0.6 nmol l^{-1} h^{-1} at Stn T37. Laminarin was hydrolyzed at all stations, at rates ranging from 1.2 to 14.6 nmol l^{-1} h^{-1}. Hydrolysis rates were highest at Stns T33 and T15s (14.2 ± 0.8 nmol l^{-1} h^{-1} and 14.6 ± 0.5 nmol l^{-1} h^{-1}), and only slightly lower at Stn T15d (12.1 ± 0.2 nmol l^{-1} h^{-1}). Laminarin hydrolysis was essentially complete after 5 d incubation at Stns T33, T15s, and T15d. Xylan was hydrolyzed at all 3 northern stations, at rates comparable to those of laminarin and chondroitin sulfate, but

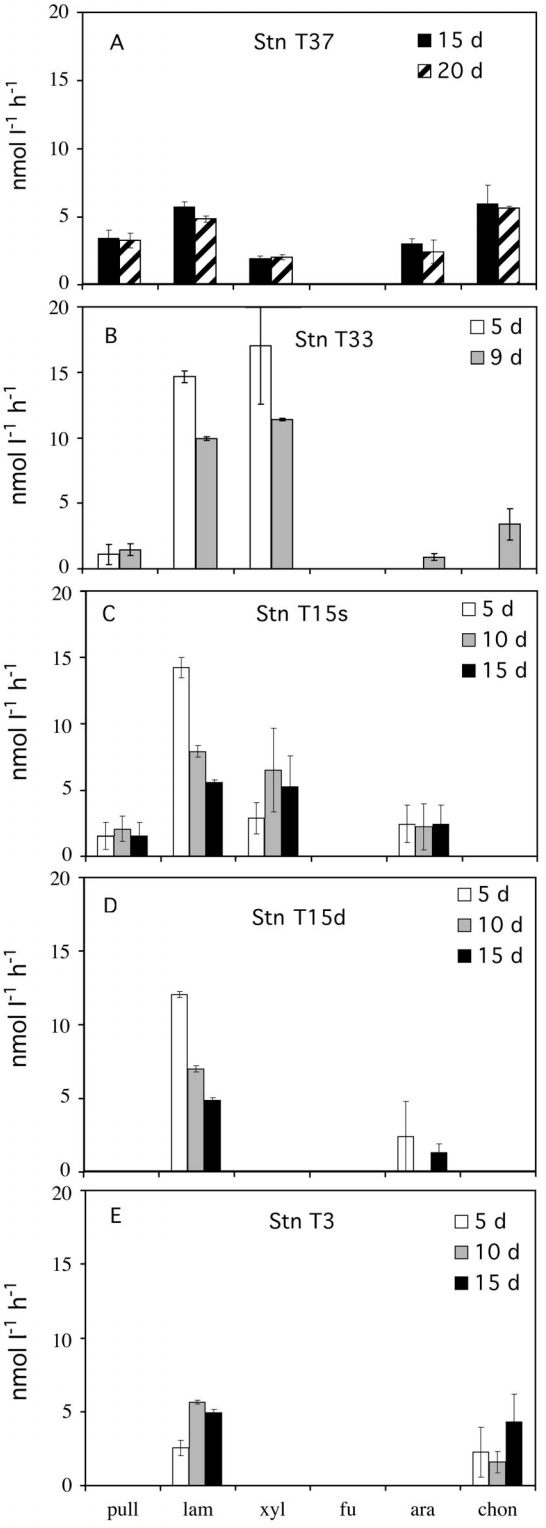

Fig. 3. Hydrolysis rates at (A) Stn T37 (24° N), (B) Stn T33 (01° N), (C) Stn T15s (23° S, surface), (D) Stn T15d (23° S, 100 m), and (E) Stn T3 (39° S). For precise station locations, see Table 1. Incubation time (d) at which hydrolysis rates were measured is shown; error bars show SD of triplicate incubations. Pull: pullulan, lam: laminarin, xyl: xylan, fu: fucoidan, ara: arabinogalactan, chon: chondroitin sulfate

was not hydrolyzed at Stns T3 or T15d. Arabinogalactan hydrolysis showed a pattern similar to that of pullulan, with rates ranging from undetectable to 3.0 ± 0.4 nmol l^{-1} h^{-1} at Stn T37. Chondroitin sulfate consistently showed a distinctive pattern: for all stations where hydrolysis was observed (except Stn T37, where the first samples were collected at 15 d), initial time points showed low or no detectable hydrolysis, and hydrolysis rates at subsequent time points increased at least 2- to 3-fold.

Microbial parameters and community composition

Cell counts, carried out at the 5 southern stations, were all on the order of 1×10^5 cells ml^{-1} (Table 1). Leucine incorporation was measured at Stns T33 and T37, and was estimated at Stns T3 and T15s based on measurements made at neighboring stations (W. H. Jeffrey et al. unpubl. data). Incorporation rates (l^{-1} seawater) decreased in the order Stn T33 > Stn T37 > Stn T3, Stn T15s.

The DGGE gel and similarity dendrogram suggested that the 6 stations compared (Stns T3, T15d, T15s, T33, T37, and AB) had distinct microbial communities (Fig. 4). One broad cluster was formed by Stns T3, T15s, and T15d; a second diverse cluster was formed by Stns T37 and T33. Stn AB appeared as an outlier from all of the southern stations.

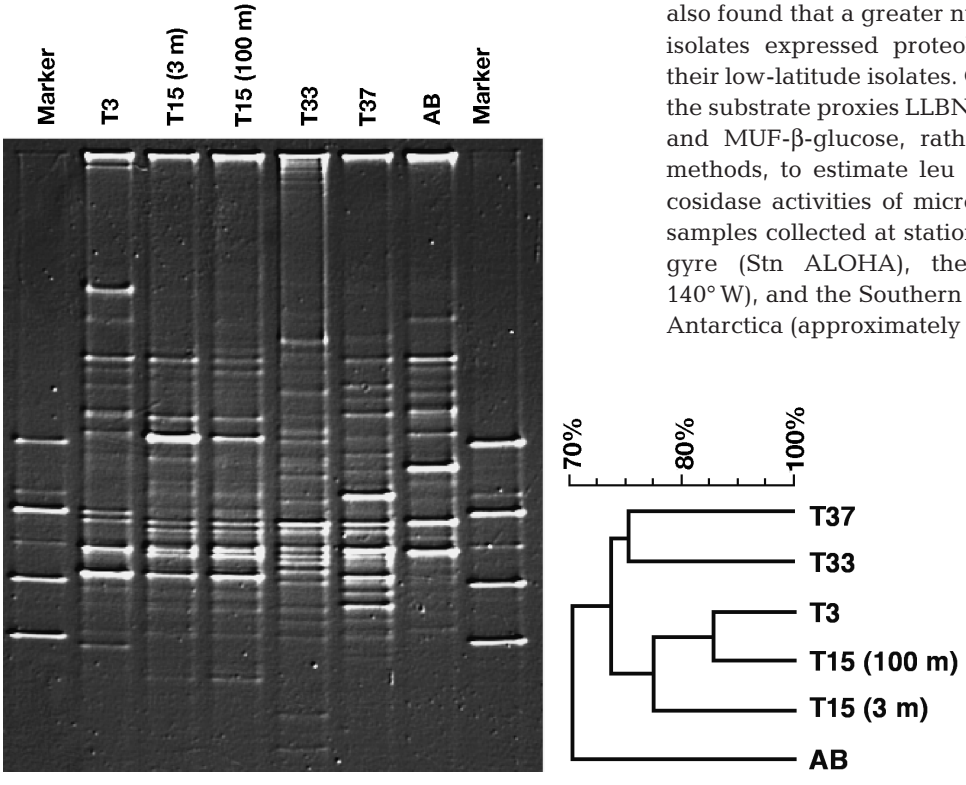

Fig. 4. Denaturing gradient gel electrophoresis of 16S rDNA PCR product from Stns T3, T15s, T15d, T33, T37 and AB. (See 'Materials and methods' for analytical conditions, Table 1 for station locations.) The similarity dendrogram shows extent of relationships among stations

DISCUSSION

Geographic patterns of enzyme activities

The common pattern of polysaccharide hydrolase activities at the 3 northernmost stations (Stns S4, AB, and J; Fig. 2) was notable, particularly since the samples were collected at different times from different water masses (see 'Materials and methods', Table 1). The more southerly stations, covering a broad range of latitudes, exhibited patterns distinctly different from the northern stations (Fig. 3). Stns T33 and T37 both displayed a broad spectrum of activities (5 of 6 substrates hydrolyzed), while Stns T15s, T15d, and T3 showed a much narrower spectrum of activities, which was, however, different from the pattern observed at the northern stations, Stns S4, AB and J. Although genetic analyses have demonstrated the occurrence of cosmopolitan microbial lineages in seawater (Mullins et al. 1995), the extent of functional similarity among seawater microbial communities is unknown. Both culture-based and culture-independent surveys have previously yielded evidence of geographic patterns in metabolic capabilities among microbes. The culture-based study of Kriss et al. (1962), for which more than 4000 heterotrophs were isolated, reported that 84% of the isolates they obtained from marine waters between 10° S and 10° N, and 77% of organisms isolated from 23° to 40° N, could hydrolyze starch. Above 40° N, only 25% of their isolates hydrolyzed starch, however. They also found that a greater number of their high-latitude isolates expressed proteolytic activity, compared to their low-latitude isolates. Christian & Karl (1995) used the substrate proxies LLBN (L-leucyl β-naphthylamine) and MUF-β-glucose, rather than culture-dependent methods, to estimate leu aminopeptidase and β-glucosidase activities of microbial communities in water samples collected at stations in the Pacific subtropical gyre (Stn ALOHA), the equatorial Pacific (0° N, 140° W), and the Southern Ocean near Palmer Station, Antarctica (approximately 64° S, 64° W). Although sea-

water LLBN activity was relatively consistent at the 3 stations, MUF-β-glucosidase activity at the equatorial station was 3 to 4 orders of magnitude greater than at ALOHA or in the Southern Ocean. They hypothesized that the relative ratios of glucosidase and peptidase activities in these samples reflect a bacterial trend with latitude in N and C utilization. Results of the current study extend these previous observations by showing evidence of geographic patterns of the activities of different polysaccharide hydrolases.

Development of substrate hydrolysis patterns

The time course over which hydrolytic activity developed at each station presumably relates to the metabolic capabilities of the microbial community at each station. As shown by Martinez et al. (1996), bacteria isolated from a single seawater sample can express distinctly different enzyme profiles. The time course differences between stations likely reflect the time required for specific biochemical processes such as induction and synthesis of enzymes, or for growth and development of particular subfractions of the microbial community. For laminarin at Stns T33, T15s, and T15d, for example, the onset of hydrolysis was rapid and hydrolysis rates were high. At these stations, presumably either laminarinase activity was relatively common among the heterotrophic microbes that produce extracellular enzymes, and/or a very active subfraction of the community produced these enzymes, or enzymes with high turnover numbers rapidly hydrolyzed the substrate. At Stns T3, S4, and AB, laminarin hydrolysis rates were somewhat slower and more constant with time. A smaller percentage of the total community may have produced laminarin-hydrolyzing enzymes, or enzyme turnover numbers might have been lower than those at Stns T33, T15s, and T15d. Rapid reactions and enzymatic hydrolysis rates might also indicate that laminarin (a storage product of diatoms; Painter 1983) was present in the water column at certain stations. The hydrolytic pattern of chondroitin sulfate is particularly interesting, since the sharp increase in hydrolysis rates with time (Figs. 2 & 3) has been consistently observed in sediments as well as seawater (Arnosti 2000, Brüchert & Arnosti 2003). Time course experiments in sediments have suggested that hydrolysis of chondroitin sulfate involves an induction lag (Arnosti 2004); in seawater, the time lag may reflect induction and/or growth of a specific portion of the microbial community.

After incubation times of days, the microbial communities sampled doubtless changed from their initial composition. The hydrolysis measurements therefore are not a 'snapshot' of the extracellular enzymes present in the water samples at the time of collection, but should be regarded as a measure of the metabolic capabilities that these microbial communities are potentially able to express. From this perspective, the fact that some of the substrates were not hydrolyzed even over extended incubation times suggests that the substrate utilization capabilities of pelagic microbial communities differ in a fundamental manner. At certain sites, key factors (perhaps a specific inducer, cofactor, or nutrient) or key metabolic capabilities are missing. The lack of hydrolysis of arabinogalactan at 4 stations, of pullulan at 5 stations, and of fucoidan at all stations, suggests that some soluble substrates are simply unavailable to certain pelagic microbial communities on timescales of days to weeks, at minimum.

Implications for cycling, composition, and bioavailability of DOC

The observation that some soluble polysaccharides are not available to planktonic microbial communities on timescales of weeks has implications for the cycling of high-molecular-weight DOC in the water column, since carbohydrates comprise a significant percentage of DOC (Benner et al. 1992). Carlson et al. (1994) have shown that DOC production and consumption in the surface ocean can be decoupled on seasonal timescales, with 'semi-labile DOC' (Carlson 2002) produced during the spring bloom resisting remineralization and being removed from the euphotic zone by wintertime deep mixing events. Although little is known about the molecular composition of semi-labile DOC, Kirchman et al. (2001) reported that in the Ross Sea, approximately 45% of the semi-labile pool of DOC was comprised of polysaccharides. Recent studies of DOC composition and dynamics have also surmised that diagenetic processes, rather than DOC sources or production, are primarily responsible for determining the neutral carbohydrate component of DOC (Meon & Kirchman 2001, Amon & Benner 2003).

Determining exactly which structural or compositional features hinder microbial remineralization of specific substrates is difficult, since the ability to isolate and sequence specific polysaccharides from seawater and sediments is beyond current technical capabilities. This lack of specific knowledge about the macromolecular structures in which most marine carbohydrates occur means that any effort to infer direct connections between specific enzyme activities and the presence of particular structures in marine environments remains highly speculative. The polysaccharides whose hydrolysis was investigated in this study, however, all are known to occur in marine systems, and/or the activities of extracellular enzymes that hydrolyze these sub-

strates have been detected in marine bacteria and sediments (see 'Introduction'). The lack of pullulanase activity in the water column at Stns S4, J, and AB, in fact, contrasts with measurements of high levels of enzyme activities in sediments from these same locations (Arnosti 2000, Arnosti & Jørgensen 2003). Furthermore, pullulanase, an enzyme whose activity was measurable in the water column only at 3 stations, can also function as a debranching enzyme of starch (Antranikian 1992), a common energy storage product among marine phytoplankton (Lee 1980). The other phytoplankton energy storage product whose hydrolysis was investigated here, laminarin (Painter 1983), was hydrolyzed at every station. Skoog & Benner (1997), however, have noted that the relative abundance of combined glucose in DOC as well as POC (particulate organic carbon) increased with depth at 2 stations they studied in the equatorial Pacific, observations that underscore the point that not all glucose-containing polymers are equally bioavailable.

Fucoidan, derived from the marine algae *Fucus*, was not measurably hydrolyzed at any station, although it was slowly hydrolyzed in sediments at Stns S4, J, and AB (Arnosti 2000, Arnosti & Jørgensen 2003, C. Arnosti unpubl. data), the only stations where sediment investigations were conducted in parallel with the seawater experiments. The lack of measurable hydrolysis of fucoidan is particularly interesting in light of the fact that transparent exopolymer particles (TEP; Alldredge et al. 1993) ubiquitous in the ocean are reported to contain high concentrations of sulfated deoxysugars (Mopper et al. 1995), a description that also fits fucoidan. Other studies also suggest that some fucose-containing polymers present remineralization 'problems': an investigation of DOM derived from algae in an ice floe demonstrated that 30% of fresh DOM was degraded over a 10 d incubation period (Amon et al. 2001). Although neutral sugars constituted 36% of the DOM that was degraded, there was no detectable change in the concentration of fucose throughout the 10 d period (Amon et al. 2001). These data suggest that while fucose-containing polysaccharides may be relatively common in marine environments they are not as easily metabolized as other polysaccharides. The fact that fucose does not dominate the total carbohydrate composition of seawater DOC (e.g. Skoog & Benner 1997), however, demonstrates that fucose-containing polymers must ultimately be remineralized in the ocean.

In any case, all of the component monomers found in the polysaccharides whose hydrolysis was investigated in this study occur in marine systems. The lack of hydrolysis of pullulan, fucoidan, and arabinogalactan at the northern stations, for example, is certainly not due to a deficit of their constituents in polar waters. The concentrations of dissolved combined neutral carbohydrates measured in a transect across the Arctic, for example, averaged ca. 740 nM, generally 10 times higher than monosaccharide concentrations, and the component monomers of these combined carbohydrates included glucose and mannose (39 and 27% of total combined neutral carbohydrates), as well as fucose, galactose, rhamnose, and arabinose (13, 9, 7, and 5% of the total, respectively), and 2 other unidentified neutral sugars (Rich et al. 1997).

The 6 polysaccharides whose hydrolysis was investigated in this study include a variety of mixed, charged, N-acetylated, and sulfated components, yet they doubtless represent only a small fraction of the natural variability of polysaccharides occurring in marine systems. Nonetheless, they demonstrate that the bioavailability of these polysaccharides is directly related to their chemical structures. Critical differences determining bioavailability may include monomer composition as well as secondary or tertiary structure, as demonstrated by the fact that hydrolysis patterns of pullulan and laminarin, both soluble linear glucose polysaccharides, were entirely different. The contrasting hydrolysis patterns of pullulan and laminarin additionally demonstrate that bioavailability of a given polysaccharide can vary drastically by location. A comparison of Figs. 2 and 3 shows that it would be difficult to select a single substrate as a proxy for carbohydrate availability among these stations, since the relative levels of hydrolytic activity measured at each station varied strongly with individual substrate. As an extreme example, reporting extracellular enzymatic hydrolysis rates of polysaccharides based on laminarin or on fucoidan would lead to the conclusion that potential hydrolysis rates from 79° N to 39° S either vary only by a factor of approximately 7 (laminarin), or that polysaccharide hydrolysis in the water column is not detectable, even on timescales of weeks (fucoidan). These examples illustrates a potential limitation inherent in using single substrates, whether high or low molecular weight, to represent the behavior of entire classes of compounds.

Relationships to bulk parameters

The precise nature of the relationships between bulk parameters such as temperature, chlorophyll concentration, or total cell counts, and the activities of specific members of microbial communities are difficult to establish. Heterotrophic microbial communities include a diverse range of microorganisms with differing metabolic requirements and potentials; effectively assessing their capabilities via a few direct measurements is difficult. Because different members of these

communities fulfill metabolically distinct roles in the degradation of organic matter, a measurement sufficiently specific to provide detailed information about selected steps in the organic carbon remineralization pathway reflects only the activity of an unknown fraction of the net microbial community. For this reason, a lack of obvious correlation between bulk community or environmental parameters and measurements of specific extracellular enzyme activities is not surprising. In this study, chlorophyll a concentrations, for example, were measured at 4 stations, and decreased in the order T3 > T33 > T15s > T37, with no obvious relationship to any other parameter.

There was likewise no direct relationship between temperature and enzyme activities measured at these 8 stations. The measurements made here, however, provide no information about either the concentrations of enzymes present or about their kinetic parameters. The enzymes expressed by organisms living in colder environments may include structural features that enable them to maintain high rates of activity despite lower temperatures (Gerday et al. 1997). A high potential hydrolysis rate could also be achieved through the activities of large numbers of enzymes, or through high turnover numbers. Nevertheless, the lack of correlation between environmental temperature and potential hydrolysis rates among these diverse stations (temperature range from 4 to 30°C) is striking. For example, rates of xylan hydrolysis were comparable at S4 and T15s (at temperatures of 4 and 17.5°C, respectively), while laminarin was hydrolyzed more slowly and chondroitin sulfate more rapidly at S4 than at T15s.

There was also no direct relationship between cell numbers (Table 1) and enzyme activities. Cell counts were highest at T3, a station where only 2 of the 6 substrates were hydrolyzed — and those 2 substrates were hydrolyzed at moderate rates. At Stn T15d, cell counts were lower by a factor of 4, but 2 of 6 substrates were again hydrolyzed. Stations T33 and T37, with the broadest spectrum of activities, were intermediate in cell counts. Leucine incorporation on a cellular basis, at the 4 stations for which data are available, likewise showed no relationship to potential enzyme activities. A lack of correlation between measurements of specific microbial activities and bulk community or environmental parameters has also been observed in other studies of microbial numbers and activities. Christian & Karl (1992), for example, reported that enzyme activities per unit microbial biomass were variable, although there was a general positive correlation between total biomass and activities. Rich et al. (1997) likewise noted that there was no significant correlation between any of the parameters of microbial activity (thymidine or leucine incorporation) and dissolved free amino acid or glucose concentrations. Likewise, they found no significant correlation between glucose turnover and leucine incorporation estimates of bacterial production.

Enzyme activities and microbial community composition

The underlying factors leading to similar enzymatic hydrolysis patterns at Stns S4, J, and AB, and the different patterns among Stns T3, T15s, T15d, T33, and T37 remain to be investigated. On a biochemical basis, differences in enzyme activity patterns could be due to differences in enzyme expression among communities fundamentally possessing the ability to hydrolyze a broad array of substrates. Failure to hydrolyze substrates such as pullulan, fucoidan, and arabinogalactan could in such a case be related to the lack of a specific inducer required to initiate expression of specific enzymes.

The patterns seen here could also be the result of a fundamental difference in microbial community composition among Stns T3, T15s, T15d, T33, T37, and the northern stations. This possibility is supported by a recent study that also used DGGE and concluded that the microbial community of the Arctic Ocean is diverse and distinct from communities found in temperate and tropical waters (Bano & Hollibaugh 2002.) Selje et al. (2004) also recently reported evidence for biogeographical patterns in planktonic microbial community composition, based on analysis of samples from a wide range of oceanic locations. The DGGE analysis of community DNA in the present study is consistent with this interpretation, as community DNA from 6 stations yielded distinctly different patterns (Fig. 4). Although DGGE analyses provides only a non-quantitative 'fingerprint' of the prevalent microbial species in a given sample, the differences among the patterns produced by samples from the southern stations were substantial, and the sole northern station included in this analysis (Stn AB) was even more distant to the southern group. Such distinct microbial communities may also differ in the type and nature of extracellular enzymes that they can express, and therefore in the nature of high-molecular-weight DOC that they can access as substrates. Variability in polysaccharide hydrolysis as observed in this study might then be directly related to microbial community composition. Genetic control of extracellular enzyme expression among marine microbes in pure cultures is only beginning to be understood (e.g. Baty III et al. 2000a,b). Expanding these investigations to encompass the responses of mixed microbial communities will be very challenging. Nevertheless, establishing direct connections between the composition of microbial communi-

ties and specific metabolic activities that they carry out will yield new insight into the factors controlling carbon cycling in marine systems. Developing the experimental means to directly link specific measurements of microbial enzymatic activity with the identity of particular organisms is an obvious research need.

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