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# Diversity and Metabolism of Marine Bacteria Cultivated on Dissolved DNA▽†

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**Dissolved DNA (dDNA) is a potentially important source of energy and nutrients in aquatic ecosystems. However, little is known about the identity, metabolism, and interactions of the microorganisms capable of consuming dDNA. Bacteria from Eel Pond (Woods Hole, MA) were cultivated on low-molecular-weight (LMW) or high-molecular-weight (HMW) dDNA, which served as the primary source of carbon, nitrogen, and phosphorus. Cloning and sequencing of 16S rRNA genes revealed that distinct bacterial assemblages with comparable levels of taxon richness developed on LMW and HMW dDNA. Since the LMW and HMW dDNA used in this study were stoichiometrically identical, the results confirm that the size alone of dissolved organic matter can influence bacterial community composition. Variation in the growth and metabolism of isolates provided insight into mechanisms that may have generated differences in bacterial community composition. For example, bacteria from LMW dDNA enrichments generally grew better on LMW dDNA than on HMW dDNA. In contrast, bacteria isolated from HMW dDNA enrichments were more effective at degrading HMW dDNA than bacteria isolated from LMW dDNA enrichments. Thus, marine bacteria may experience a trade-off between their ability to compete for LMW dDNA and their ability to access HMW dDNA via extracellular nuclease production. Together, the results of this study suggest that dDNA turnover in marine ecosystems may involve a succession of microbial assemblages with specialized ecological strategies.**

Bacteria help regulate rates of organic matter mineralization, nutrient cycling, and energy transfer in aquatic environments (4). These ecosystem processes are commonly limited by the quantity and quality of resources, such as dissolved organic matter (DOM) (3, 23). One acknowledged, but possibly underappreciated, source of DOM in aquatic ecosystems is DNA. Dissolved DNA (dDNA) is produced by cell death, lysis, and excretion (20, 31, 41) and is an important component of the DOM pool in marine environments. For example, water column concentrations of dDNA often exceed particulate DNA concentrations (8), while extracellular DNA in marine sediments represents the largest reservoir of DNA in the world ocean (11). The turnover rates for dDNA are rapid (<1 day), suggesting that it may be a high-quality resource capable of supporting microbial metabolism (31). dDNA helps bacteria meet their carbon and nitrogen demands (18, 22) and is a source of nucleotides that can be directly used for DNA and RNA synthesis (29, 35). Due to its elemental composition, dDNA may be an especially valuable source of phosphorus. For example, dDNA accounts for up to 25% of the total phosphorus in some freshwater ecosystems (38), and it is estimated to provide one-half of the phosphorus required for benthic bacterial biomass production in some marine ecosystems (10).

The nutritional importance of dDNA in aquatic ecosystems ultimately depends on the ability of microorganisms to take up this macromolecule. It is generally accepted that bacteria are constrained by their inability to passively transport materials with >0.6 kDa molecular masses across their cell walls (40). Environmental dDNA is much larger than this; its molecular mass ranges from 6 to 23,000 kDa (24). Therefore, bacteria have evolved different strategies for taking up dDNA. Some marine bacteria are capable of degrading dDNA with extracellular nucleases and then consume the hydrolyzed products (10, 29). Alternatively, some bacteria are naturally competent and take up intact dDNA for nutritional and genetic purposes via complex pathways consisting of specialized proteins (5, 15). In aquatic ecosystems, the size of DOM has a strong effect on the diversity and metabolism of aquatic microorganisms. For example, some groups of marine bacteria are specialized at using different types of low-molecular-weight (LMW) DOM (6), and manipulation of DOM size fractions has been shown to alter the composition of estuarine bacterial communities (7). Interactions between dDNA size and metabolic strategies of different bacteria, therefore, may ultimately influence the rates of dDNA turnover and the patterns of microbial diversity in marine ecosystems.

Despite the potential importance of dDNA as a microbial resource capable of influencing ecosystem processes, very little is known about the diversity, phylogenetic identity, or interactions of heterotrophic bacteria that consume dDNA. In this study, experiments with water from Eel Pond (Woods Hole, MA) were conducted to characterize the diversity of cultivable marine bacteria capable of using dDNA as the primary source of carbon, nitrogen, and phosphorus. Furthermore, I tested the hypothesis that dDNA size influences bacterial metabolism by comparing the abundance, growth, and nuclease production of bacteria exposed to LMW dDNA and high-molecular-weight

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(HMW) dDNA. Understanding the ecological strategies of marine bacteria involved in dDNA degradation should provide new insight into microbial species interactions and the factors influencing the biogeochemical processing of dDNA in aquatic ecosystems.

## MATERIALS AND METHODS

**Field sampling.** Water samples were obtained from Eel Pond, a small impoundment open to Great Harbor in Woods Hole, MA. A peristaltic pump was used to collect water from three depths (0.5, 2.5, and 5.0 m) at a sampling location with a maximum depth of 5.5 m. Water samples were pooled and transported to the laboratory immediately. In the field, temperature, salinity, dissolved oxygen content, and conductivity were measured with a YSI multiprobe water quality checker. The abundance of bacteria in the pooled water samples was determined by 4′,6′-diamidino-2-phenylindole (DAPI) staining and epifluorescence microscopy. The dDNA concentration in the pooled sample was determined spectrofluorimetrically using SYBR green I (27) and standard solutions of *Escherichia coli* DNA (Sigma).

**dDNA media.** The DNA used was double-stranded DNA derived from salmon testes (D-1626; sodium salt; Sigma). Using this DNA, HMW and LMW dDNA media were created. To obtain HMW dDNA, 1 g of DNA was dissolved in 100 ml of distilled water. To obtain LMW dDNA, 1 g of DNA was dissolved in 100 ml of distilled water with 7,000 U of DNase I (104 132; Roche) in DNase buffer (200 μM Tris [pH 8.3], 500 μM KCl, 10 μM $MnCl_2$) at 37°C for 3 h with continuous stirring. The DNase reaction was stopped by heating the preparation at 70°C for 20 min. Deactivated DNase (100°C for 1 h) and DNase buffer were added to the HMW dDNA to control for the compounds that were used to obtain LMW dDNA. Agarose gel electrophoresis was used to confirm that the DNase treatment altered the size of the original DNA source; HMW dDNA was ≤10 kb long, while LMW DNA was ≤250 bp long (data not shown).

HMW or LMW dDNA was then added to sterile artificial seawater medium at a final concentration of 1 g DNA/liter. The seawater medium contained 30 g/liter of sea salts (59883; Sigma), 10 mM HEPES buffer, and 1 ml of a trace element stock. The trace element stock contained (dissolved in 1 liter of distilled $H_2O$) 5.2 g EDTA, 2.1 g $FeSO_4$, 30 mg $H_3BO_3$, 100 mg $MnCl_2 \cdot 4H_2O$, 190 mg $CoCl_2 \cdot 6H_2O$, 1 g $NiCl_2 \cdot 6H_2O$, 2 mg $CuCl_2 \cdot 2H_2O$, 144 mg $ZnSO_4 \cdot 7H_2O$, 36 mg $Na_2MoO_4 \cdot 2H_2O$, 6 mg $Na_2SeO_3 \cdot 5H_2O$, and $Na_2WO_4 \cdot 2H_2O$. Although HEPES is a buffer that contains organic carbon and organic nitrogen, control experiments demonstrated that marine *Vibrio* strains could not grow on HEPES in the absence of dDNA (data not shown). In attempt to reduce organic contamination, washed agar (0.75%) was added to the seawater medium prior to autoclaving. The washing process consisted of rinsing the agar seven times with distilled water, once with 70% ethanol, and once with acetone, followed by aeration at 40°C for 3 days. The control medium consisted of all the ingredients described above except DNA.

**Enrichments.** Dilutions of the pooled Eel Pond sample (100 μl of $10^0$ to $10^{-4}$ dilutions) were plated onto replicate agar plates containing HMW dDNA, LMW dDNA, or no dDNA (control). The plates were incubated at 30°C in the dark and monitored daily for colony formation. After 6 days, a subset of replicate plates was used for plate wash PCR (see below). After 10 days, bacterial abundance was estimated by calculating the total number of CFU for a set of replicate plates for each of the three dDNA treatments. One-way analysis of variance (ANOVA) and Tukey's HSD (honestly significant difference) were used to test for differences in CFU among DNA treatments.

**Diversity of dDNA-consuming bacteria.** Clone libraries for both HMW and LMW dDNA enrichments were constructed by extracting genomic DNA from agar plates using a plate wash technique (39) and an Ultraclean fecal DNA isolation kit (MoBio Laboratories). 16S rRNA genes were amplified using universal bacterial primers 8f and 1492r, and the resulting PCR products were cloned using an Invitrogen TOPO TA cloning kit. A total of 106 clones were picked for sequencing (40 LMW dDNA clones and 66 HMW dDNA clones). Purified plasmids were used as templates for partial sequence determination using bacterial 16S rRNA gene primer 531R (5′-TAC CGC GGC TGC TGG CAC-3′) with a BigDye Termination kit, version 3.0 (Applied Biosystems). Completed-reaction mixtures were examined with a 96-capillary 3730xl DNA analyzer (Applied Biosystems). Sequences were aligned using the ARB phylogenetic software package (25). Sequences were checked for chimeras using Bellerophon (17). DOTUR (36) was used to estimate taxon richness, assuming a 99% sequence similarity cutoff. Finally, ∫-LIBSHUFF (37) was used to test the null hypothesis that the HMW and LMW dDNA clone libraries came from a common population.

**Phylogenetic identity of isolates.** Single-colony isolates from HMW and LMW dDNA plates were identified by direct sequencing of 16S rRNA genes. Isolates were randomly selected from the enrichments and replated onto the original dDNA medium. Individual colonies were lysed by boiling them in 20 μl of 0.05% Triton X-100 for 5 min. One microliter of each resulting supernatant was used as a template in a 25-μl PCR mixture. The 37 PCR products were screened by agarose gel electrophoresis and sequenced (see above) after they were cleaned with an ExoSAP-IT kit (USB Corporation). Phylogenetic identification of the sequences was performed by determining the most closely related representatives in the BLAST database (www.ncbi.nlm.nih.gov/BLAST). A total of 37 isolates were identified (18 isolates from HMW dDNA plates and 19 isolates from LMW dDNA plates).

**Growth curves with dDNA.** The 19 phylogenetically identified isolates from LMW dDNA plates were used in a set of growth curve experiments performed with liquid dDNA media. These experiments were performed in order to characterize bacterial growth on different sizes of dDNA. The liquid media used were the media described above without agar. For each isolate, there were six test tubes (15 ml); three replicate tubes contained LMW dDNA, and three replicate tubes contained HMW dDNA. The tubes were inoculated with a homogeneous suspension of cells (100 μl of a 0.9% NaCl suspension) and incubated in an upright shaker at 30°C. Growth was monitored by determining the change in absorbance at 600 nm over time (110 h). The effect of dDNA type (HMW dDNA versus LMW dDNA) on bacterial growth over time was assessed using repeated-measures ANOVA (SAS PROC MIXED with an AR-1 covariance matrix). A significant effect of time in the repeated-measures ANOVA model indicated that growth changed during the experiment irrespective of the dDNA treatment. A significant effect of dDNA indicated that growth with LMW dDNA and growth with HMW dDNA were different irrespective of time. A significant interaction (time × dDNA) indicated that changes in growth over the course of the experiment were affected by the dDNA treatment.

**dDNA degradation.** dDNA degradation is one of the first steps involved in the ability of bacteria to use dDNA as a resource. I developed an assay to evaluate the abilities of different bacterial isolates to degrade HMW dDNA. This assay is similar to the single radial enzyme diffusion method, in which a nucleic acid stain (e.g., SYBR green or ethidium bromide) is used to quantify the clearing of DNA on agarose gel plates due to DNase activity (42). dDNA degradation was assayed for 64 isolates (32 LMW dDNA isolates and 32 HMW dDNA isolates), 19 and 18 of which were identified phylogenetically, respectively. For each isolate, a small amount of cell biomass was aseptically transferred into 250 μl of 0.9% NaCl after a single colony was lightly touched with the tip of a toothpick. In duplicate, 50 μl of a cell suspension was spotted in the middle of 100-mm-diameter petri dishes containing HMW dDNA agar (see above). The petri dishes were incubated at 30°C for 10 days and were then soaked in a 1× Tris-borate-EDTA bath with ethidium bromide (100 μg $liter^{-1}$) for 2 min. The soaked plates were rinsed in a 1× Tris-borate-EDTA bath for 1 min before images were obtained under UV light with a transilluminator using image analysis software. The positive controls consisted of petri dishes in which 50 μl (7,000 U) of DNase I was added to HMW dDNA agar. The area of DNA degradation relative to the area of the entire petri dish was determined using AxioVision software (version 4.4). Percentages were then converted to rates of dDNA degradation by assuming that zones of clearing were completely depleted of HMW dDNA. Student *t* tests were used to test the null hypothesis that isolates from different enrichments degraded the same amount of HMW dDNA.

## RESULTS

**Field characteristics.** The salinity, temperature, and conductivity were uniform throughout the water column in Eel Pond. In contrast, the dissolved oxygen concentration decreased with depth from 7.3 to 3.6 mg $liter^{-1}$. The concentration of DAPI-stained bacteria in the pooled water sample was $1.3 \times 10^7 \pm 0.71 \times 10^7$ cells $ml^{-1}$ (average ± standard error). The dDNA concentration in the pooled water sample was 26 ± 0.3 μg $liter^{-1}$ (mean ± standard error).

**Diversity of dDNA-consuming bacteria.** Using the 106 sequences generated from the plate wash PCR, 32 taxa were identified with a 99% sequence similarity cutoff. The taxon richness of the LMW dDNA clone library and the taxon richness of the HMW dDNA clone library were comparable (18

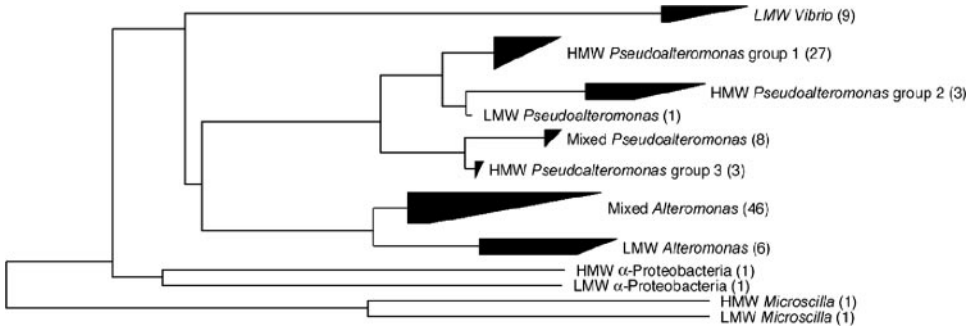


FIG. 1. Unrooted phylogenetic tree for 16S rRNA gene sequences from bacterial assemblages that developed on LMW and HMW dDNA. The numbers in parentheses are the numbers of clones in clades.

and 21 taxa, respectively). However, only 7 of the 32 taxonomic groups (22%) contained sequences obtained from treatments; the remaining 25 taxonomic groups (78%) contained sequences obtained with either LMW dDNA or HMW dDNA. ∫-LIBSHUFF analysis indicated that the compositions of the clone libraries were significantly different ($P = 0.0001$). Most (88%) of the sequences belonged to the γ-proteobacteria and were represented by *Vibrio* spp., *Alteromonas* spp., and *Pseudoalteromonas* spp. Two sequences belonged to the α-proteobacteria; one of these sequences was closely related to a *Roseobacter* sp. (99% similarity), and the other was most closely related to *Kordiimonas gwangyangensis* (90% similarity). In addition, two sequences belonged to the *Bacteroidetes*; one of these sequences was closely related to a *Flexibacter* sp. (95% similarity), and the other was most closely related to *Microscilla arenaria* (94% similarity). Some sequences formed monophyletic groups with respect to the type of dDNA. For example, *Vibrio* sequences were recovered only when LMW dDNA was used. In addition, there were three HMW *Pseudoalteromonas* clades and a single LMW *Alteromonas* clade. The largest clade was the "mixed" *Alteromonas* group, which comprised 17 sequences obtained with LMW dDNA and 29 sequences obtained with HMW dDNA (Fig. 1).

**Bacterial abundance with LMW and HMW dDNA.** The average number of CFU was higher on LMW dDNA agar plates than on HMW dDNA agar plates ($5.2 \times 10^5 \pm 0.32 \times 10^5$ cells $ml^{-1}$ versus $2.1 \times 10^5 \pm 0.43 \times 10^5$ cells $ml^{-1}$; $P < 0.0001$, as determined by ANOVA) (Fig. 2). There were significantly fewer CFU on control plates ($1.5 \times 10^4 \pm 0.99 \times 10^4$ cells $ml^{-1}$) than on both LMW dDNA and HMW dDNA agar plates ($P < 0.0001$, as determined by ANOVA).

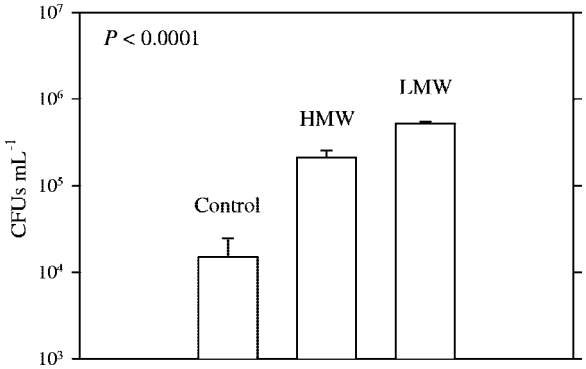


FIG. 2. CFUs from enrichments containing no added DNA (i.e., control), HMW dDNA, and LMW dDNA. The data are means ± standard errors of the means. The *P* value was determined by a one-way ANOVA.

**Growth curves with dDNA.** A majority (58%) of the bacteria that were isolated from the LMW dDNA enrichments were affected by the type of dDNA in the growth curve experiments (Table 1). In all but one instance (*Pseudoalteromonas* isolate 5), isolates obtained from the LMW dDNA enrichments had higher levels of growth in the presence of LMW dDNA than in the presence of HMW dDNA (Table 1 and Fig. 3). The remaining isolates (42%) exhibited similar growth in the presence of LMW dDNA and HMW dDNA.

**dDNA degradation.** Degradation of dDNA by marine bacteria was readily apparent when the ethidium bromide assay was used. For the negative control, dDNA fluoresced evenly across the agar surface (Fig. 4A), whereas distinct clearing

TABLE 1. Results of repeated-measures ANOVA for bacterial growth on dDNA[a]

| Isolate | *P* | Effect |
|---|---|---|
| *Vibrio* isolate 1 | 0.0005 | DNA × time |
| *Vibrio* isolate 2 | 0.0009 | DNA × time |
| *Vibrio* isolate 3 | 0.0003 | DNA × time |
| *Pseudoalteromonas* isolate 1 | >0.05 | NS |
| *Pseudoalteromonas* isolate 2 | >0.05 | NS |
| *Pseudoalteromonas* isolate 3 | 0.02 | DNA × time |
| *Pseudoalteromonas* isolate 4 | 0.0001 | DNA × time |
| *Pseudoalteromonas* isolate 5 | 0.0004 | DNA × time |
| *Pseudoalteromonas* isolate 6 | 0.02 | Time |
| *Pseudoalteromonas* isolate 7 | 0.05 | DNA |
| *Alteromonas* isolate 1 | <0.001 | DNA × time |
| *Alteromonas* isolate 2 | >0.05 | NS |
| *Alteromonas* isolate 3 | 0.004 | Time |
| *Alteromonas* isolate 4 | >0.05 | NS |
| *Alteromonas* isolate 5 | 0.004 | DNA × time |
| *Alteromonas* isolate 6 | 0.008 | Time |
| *Alteromonas* isolate 7 | >0.05 | NS |
| *Microscilla* isoalte 1 | 0.04 | DNA × time |
| *Microscilla* isolate 2 | 0.001 | DNA × time |

[a] Nineteen strains from the LMW dDNA enrichments were isolated and phylogenetically identified by 16S rRNA gene sequencing. These isolates were then grown in liquid medium containing either LMW or HMW dDNA for 110 h. Bacterial growth was measured by determining the absorbance at 600 nm. Significant *P* values are shown for the highest-order effect (i.e., DNA × time) and for significant main effects (DNA or time). NS, not significant.

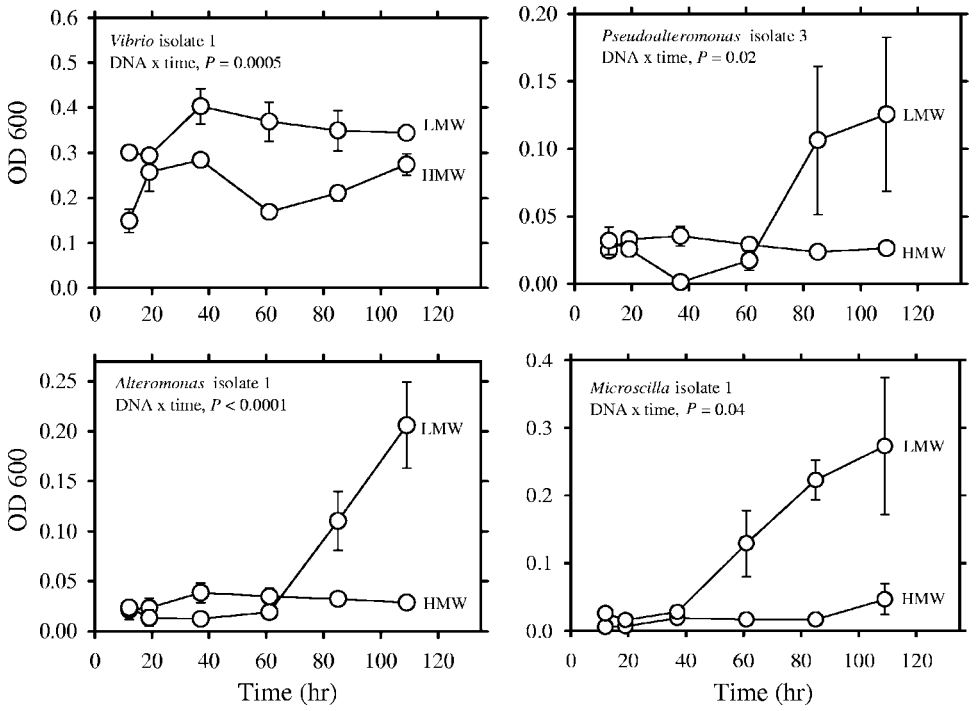


FIG. 3. Representative growth curves for some of the major groups of marine bacteria enriched on dDNA. Bacterial strains were originally isolated from LMW dDNA enrichments and then inoculated into either LMW or HMW dDNA liquid medium. The data are means ± standard errors of the means. The *P* values were determined by repeated-measures ANOVA. Note that the scales on the *y* axes are different in different panels. OD 600, optical density at 600 nm.

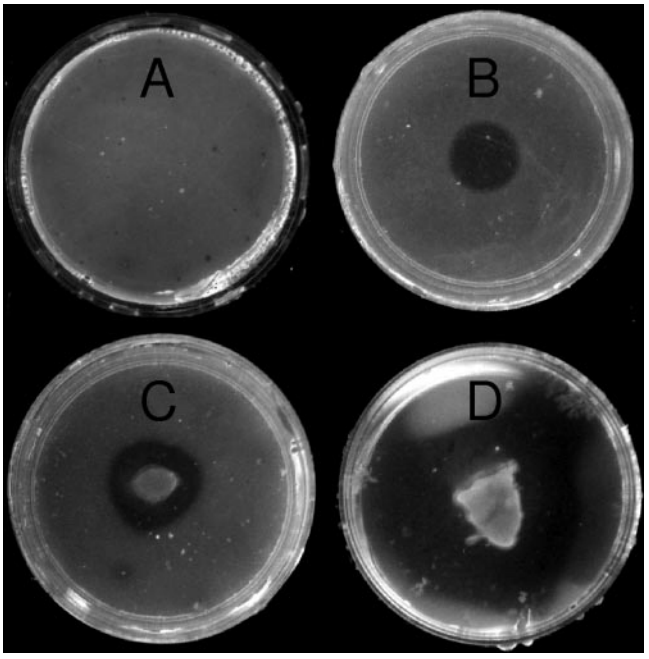


FIG. 4. Ethidium bromide assay used to document HMW dDNA degradation. (A) Negative control stained with ethidium bromide; (B) positive control with a 50-μl drop of DNase I spotted in the middle of the plate, followed by ethidium bromide staining; (C) bacterial activity resulting in 13% HMW dDNA degradation; (D) bacterial activity resulting in 65% HMW dDNA degradation. The images in panels C and D were obtained after ethidium bromide staining of HMW dDNA plates which had incubated for 10 days following spotting of a cell suspension in the center of each plate.

zones formed around the regions where DNase I was applied as a positive control (Fig. 4B). Bacterial growth was confined to a small central region of the plate where the inoculum was spotted. Bacterial biomass was detected as small fluorescent regions (particulate DNA) surrounded by zones of dDNA clearing (Fig. 4C and D). Bacteria originally isolated from HMW dDNA enrichments degraded twice as much HMW dDNA as bacteria originally isolated from LMW dDNA enrichments ($14 \pm 2.0$ versus $7 \pm 2.0$ mg liter$^{-1}$ day$^{-1}$; $P = 0.015$, as determined by a *t* test) (Fig. 5). I determined whether this relationship held for different phylogenetic groups of bacteria that occurred in both of the dDNA treatments. The rates of HMW dDNA degradation were not different for *Pseudoaltero-*

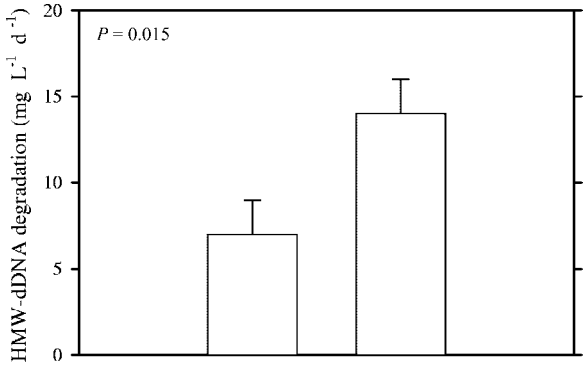


FIG. 5. Rates of HMW dDNA degradation determined by the ethidium bromide assay for strains originally isolated from LMW and HMW dDNA enrichments. The *P* value was determined by a *t* test.

*monas* spp. ($P = 0.5$, as determined by a *t* test), but *Alteromonas* spp. isolated from HMW dDNA enrichments degraded three times more DNA than *Alteromonas* spp. isolated from LMW dDNA enrichments ($15 \pm 3.0$ versus $5 \pm 3.0$ mg liter$^{-1}$ day$^{-1}$; $P = 0.04$, as determined by a *t* test).

## DISCUSSION

dDNA is a ubiquitous component of the DOM pool in nearly all aquatic environments (19, 38). The biogeochemical cycling of dDNA largely depends on microbial activity (31). dDNA may be a high-quality resource capable of supporting aquatic microbial metabolism, yet very little is known about the identity and physiology of dDNA-consuming bacteria. Some, but not all, of the marine microbes isolated in this study were able to take up and efficiently use dDNA as the primary source of energy and nutrition. Bacterial isolates typically exhibited less growth on HMW dDNA than on LMW dDNA, especially if they were originally isolated from LMW dDNA enrichments. This difference in bacterial growth may reflect energetic costs associated with nuclease production or, in some cases, an inability to produce nucleases altogether (2). The results of this study suggest that dDNA size can influence microbial diversity, but they also imply that different bacterial species may specialize on certain size fractions of the dDNA pool. Thus, dDNA turnover in marine ecosystems may involve a succession of microbial assemblages with different ecological strategies.

**Diversity of dDNA-consuming bacteria.** A relatively diverse assemblage of marine bacteria was cultivated in this study, especially considering that a single molecule was used as the primary source of carbon, nitrogen, and phosphorus. Members of the *Bacteroidetes* and α-proteobacteria comprised 12% of the taxa in the clone libraries, while the remainder were members of the γ-proteobacteria. The latter group was dominated by *Alteromonas* and *Pseudoalteromonas*, which are well known for their ability to degrade HMW compounds via extracellular enzyme production (9, 28).

However, culture-independent surveys have shown that the abundance of γ-proteobacteria is generally low compared to other groups of bacteria in marine ecosystems (16). Despite being relatively rare, the γ-proteobacteria comprise an ecologically important group of microbes that tend to be fast growing under resource-rich conditions. Marine systems tend to experience episodic pulses of high resource availability due to both large-scale processes (e.g., upwelling, eddies, or bloom dynamics) and small-scale processes (e.g., sloppy grazing and viral lysis). Some studies have suggested that the γ-proteobacteria are able to take advantage of these resource pulses. For example, in southern California, the abundance of γ-proteobacteria increased in response to a dinoflagellate bloom (14) and experimental DOM enrichment (32). Due to the biological controls on its production (31, 20, 41), dDNA may be an example of the type of resource that helps opportunistic bacteria, like the γ-proteobacteria, persist over time in marine environments. It is important to note that dDNA consumption is most likely not restricted to the γ-proteobacteria or other taxa found in this study. The cultivation approach used here may provide only a glimpse of the true diversity of microbes capable of using dDNA as a source of energy or nutrition in marine environments.

**dDNA size affects microbial composition.** dDNA size had a strong effect on the composition of the bacteria represented in the clone libraries. Initially, I predicted that bacteria which grew on HMW dDNA would be a subset of the bacterial assemblage which grew on LMW dDNA. Such an outcome would be consistent with a loss of taxon richness due to the inability of some bacteria to efficiently take up and use HMW dDNA. In contrast to the initial prediction, the taxon richness obtained with LMW dDNA and the taxon richness obtained with HMW dDNA were comparable. The bacterial composition, however, was significantly affected by dDNA size. Only 22% of the taxa were capable of using both types of dDNA, while the vast majority of bacteria were limited to growing on either LMW or HMW dDNA. Additional effects of dDNA size on microbial composition were apparent from the structure of the phylogenetic tree constructed from the clone libraries. For example, a number of *Pseudoalteromonas* groups were monophyletic with respect to HMW dDNA, while *Vibrio* clones were only recovered from the LMW dDNA treatment (Fig. 1).

Other studies have also shown that DOM size affects aquatic microbial composition. An estuarine bacterial community from a blackwater river was strongly influenced by exposure to different size fractions of freshwater DOM (7). Although the estuarine bacterial community was affected by substrate size, it may have also been responding to variation in the chemical composition of the different DOM size fractions. For example, the composition of the bacterial community after DOM additions in the Delaware Bay was dependent on the type of HMW molecule investigated (6). In contrast, the only apparent factor in this study that could account for treatment-level differences in microbial composition was the size or structure of the dDNA molecule.

**Bacterial growth affected by dDNA size.** The changes in microbial composition corresponded to trends in the abundance and growth of bacteria on LMW and HMW dDNA. First, bacterial abundance was approximately 2.5 times greater on LMW dDNA plates than on HMW dDNA plates (Fig. 2). Second, one-half of the strains isolated from LMW dDNA enrichments exhibited reduced growth when they were inoculated into HMW dDNA liquid medium (Table 1 and Fig. 3). Together, these results demonstrate that dDNA metabolism varies among marine bacteria but in general is strongly affected by dDNA size. These findings are consistent with the view that DOM uptake by bacteria is constrained by their ability to take up HMW molecules (6). The results of the growth curve experiments also provide insight into a potential mechanism that may have contributed to the observed differences in microbial composition. Specifically, bacteria isolated from the LMW dDNA enrichments generally performed better when they were grown in LMW dDNA liquid medium (Table 1 and Fig. 3) and thus may have outcompeted HMW strains under these conditions.

**DNA degradation varies among marine bacteria.** The results of the ethidium bromide assay clearly showed that bacteria isolated from different enrichments degraded dDNA at different rates. For example, many of the isolates originating from the LMW dDNA enrichment degraded <1% of the available HMW dDNA, suggesting that they lacked the ability to produce extracellular nucleases. In contrast, bacteria isolated from the HMW dDNA enrichments degraded HMW dDNA two

times faster than bacteria isolated from the LMW dDNA enrichments (Fig. 5).

The ethidium bromide assay, which has not been used previously to assess dDNA degradation by marine bacteria, is similar to assays used for measuring nuclease activity (42, 12). Therefore, it is reasonable to assume that the DNA degradation observed in this study reflected some integrated features of bacterial nuclease production, which might include variation in specific activities, production rates, or diffusive properties of different enzymes. Moreover, the results of the DNA degradation assay could be influenced by the physical location of the enzymes (in particular, whether nucleases tend to be bound to the cell surface or released freely into the environment).

Results of this study indicate there may be substantial variation in dDNA degradation even among closely related taxa. For example, *Pseudoalteromonas* spp. degraded DNA at comparable rates regardless of the enrichment conditions. In contrast, the dDNA degradation rates were three times greater for *Alteromonas* spp. isolated from HMW dDNA enrichments than for *Alteromonas* spp. isolated from LMW dDNA enrichments, suggesting that observed "microdiversity" may translate into phenotypic diversity. It is important to note, however, that degradation is only the first step involved in dDNA metabolism. The overall metabolism of dDNA-consuming bacteria should also be influenced by factors such as uptake kinetics and yield, which are reflected in growth dynamics (Fig. 3).

Currently, two models are used to explain bacterial uptake of dDNA for nutritional purposes. According to the first model, some bacteria are naturally competent and capable of taking up intact macromolecular DNA (13). Competency requires proteins related to type IV pili and type II secretion systems, in addition to cell surface ectonuclease production (5). Competency often results in natural transformation, a mechanism for horizontal gene transfer, which may be important for explaining the evolutionary history of some marine bacteria (33). Competency has also been suggested to be a potential nutritional strategy for some bacteria (34, 15), but it has not been well documented for marine microbes. One of the few studies on this topic found that estuarine bacteria were ineffective at transporting intact gene sequences from the environment into the cell (29). Such findings support the second model, whereby bacteria degrade dDNA extracellularly with the aid of nucleases and then consume the hydrolyzed products (26, 29). Early work on this question suggested that many bacteria isolated from marine environments, including *Pseudomonas* spp., *Flavobacterium* spp., *Vibrio* spp., and *Acinetobacter* spp., are capable of using this DNA consumption strategy (26). Although the current study was not designed to differentiate the mechanisms of dDNA uptake in marine bacteria, the results may shed some light on this topic. For example, dDNA degradation assays revealed wide zones of clearing around confined regions of bacterial biomass (Fig. 4C and D). This pattern of dDNA uptake is more consistent with extracellular enzyme degradation (26) than with competency, which requires binding of DNA by the bacterial cell (5). Thus, the results of the current study support the model involving extracellular dDNA degradation followed by uptake of the hydrolyzed products (29), but additional research is needed to better understand the relative importance of extracellular enzyme hydrolysis and competency strategies in dDNA uptake by marine bacteria.

**Ecological implications.** Results obtained in this study indicated that populations of co-occurring marine bacteria were specialized for using either LMW or HMW dDNA. On average, bacteria isolated from LMW dDNA enrichments exhibited greater growth on LMW dDNA than on HMW dDNA (Fig. 3 and Table 1), while bacteria isolated from HMW dDNA enrichments were more effective at enzymatic degradation of HMW dDNA than bacteria isolated from LMW dDNA enrichments (Fig. 5). This type of resource specialization on different sizes of dDNA suggests that there is a trade-off which could help maintain microbial diversity (21) and influence ecosystem processes. For example, resource specialization by distinct groups of bacteria in a microbial assemblage has been shown to influence rates of DOM consumption in marine ecosystems (6).

Some features of the dDNA experimental system are similar to features of a recent simulation model that was used to examine the interactions between microbial populations receiving HMW organic substrates (1). In this model there were two types of bacteria: bacteria that were directly responsible for HMW substrate degradation via extracellular enzymes ("producers") and bacteria that took advantage of the extracellular enzymes generated by producers ("cheaters"). Simulations revealed that cheaters had a selective advantage when the enzyme cost increased for producers. In contrast, producers were favored in heterogeneous environments or when enzymes remained close to the producer's cell surface. Ultimately, the model predicted that producers and cheaters would coexist at intermediate levels of enzyme cost and intermediate levels of enzyme diffusivity. The results of this experimental study suggest that the dDNA system may be ideal for testing theoretical predictions about extracellular enzyme activity, microbial interactions, and DOM dynamics.

Previous studies have documented that there is rapid uptake of dDNA by aquatic microbial communities, suggesting that dDNA is an important component of DOM in freshwater and marine ecosystems (30, 31). Despite this, very little is known about the identity and metabolism of environmental bacteria capable of using dDNA as a source of energy or nutrition. Using a novel cultivation approach, this study provides an initial assessment of the diversity of dDNA-consuming bacteria in marine ecosystems. Almost certainly, the list of dDNA-consuming microbes will grow with the development and application of cultivation-independent approaches. Importantly, the results obtained in this study highlight some of the ecological strategies that marine bacteria use for consuming dDNA. Moreover, the results suggest that the biogeochemical cycling of dDNA in marine ecosystems may involve a succession of microbial assemblages with different metabolic capabilities.

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