Diversity and metabolism of marine bacteria cultivated on dissolved DNA

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Submitted to Applied and Environmental Microbiology: Microbial Ecology section

Date of submission: 25 July 2006 (AEM01730-06 Version 1.0)
Decision and reviews received: 25 October 2006
Date of resubmission: 15 November 2006 (AEM02674-06 Version 1)
Decision and reviews received: 31 January 2007
Date of resubmission: 20 February 2007 (AEM02674-06 Version 2)

Running Title: dDNA consuming bacteria
ABSTRACT

Dissolved DNA (dDNA) is a potentially important source of energy and nutrients in aquatic ecosystems. Little is known, however, about the identity, metabolism, and interactions of microorganisms capable of consuming dDNA. Bacteria from Eel Pond (Woods Hole, Massachusetts) 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 taxon richness developed on LMW- and HMW-dDNA. Since the LMW- and HMW-dDNA used in this study were stoichiometrically identical, the results affirm that the size alone of dissolved organic matter (DOM) can influence bacterial community composition. Variation in the growth and metabolism of isolates provided additional insight into mechanisms that may have generated differences in bacterial community composition. For example, bacteria from LMW-dDNA enrichments generally experienced higher growth on LMW-dDNA than HMW-dDNA. In contrast, bacteria 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, results from this study suggest that dDNA turnover in marine ecosystems may involve a successional sequence of microbial assemblages with specialized ecological strategies.
INTRODUCTION

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 through cell death, lysis, and excretion (20, 31, 41) and constitutes an important component of the DOM pool in marine environments. For example, water column concentrations of dDNA often exceed particulate DNA (8), while extracellular DNA in marine sediments represents the largest reservoir of DNA in the world ocean (11).

Turnover rates of dDNA are rapid (<1 d), 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/RNA synthesis (29, 35). Due to its elemental composition, dDNA may be an especially valuable supply 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 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 > 0.6 kDa across their cell walls (40). The size of environmental dDNA is much larger, ranging from 6 – 23,000 kDa (24).

Therefore, bacteria have evolved different strategies for taking up dDNA. Some marine bacteria are capable of degrading dDNA with the aid of extracellular nucleases and then consume 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 strong effects on the diversity and metabolism of aquatic microorganisms. For example, groups of marine bacteria specialize in their ability to use different types of low molecular weight (LMW) DOM (6), and manipulations of DOM size fractions altered 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 among heterotrophic bacteria that consume dDNA. In this study, experiments with water from Eel Pond (Woods Hole, Massachusetts) were conducted to characterize the diversity of cultivable marine bacteria capable of using dDNA as the primary source of carbon, nitrogen, and phosphorus. Furthermore, the hypothesis was tested that dDNA size influences bacterial metabolism by comparing the abundance, growth, and nuclease production of bacteria exposed to both LMW and high molecular weight (HMW) dDNA. Understanding the ecological strategies of marine bacteria involved in dDNA degradation will 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, 5.0 m) at a sampling location with a maximum depth of 5.5 m. Water samples were pooled and transported immediately back to the laboratory. In the field, temperature, salinity, dissolved oxygen, and conductivity were measured with a YSI multiprobe water quality checker. The abundance of bacteria in the pooled water samples was quantified via DAPI staining and epifluorescence microscopy. dDNA concentration of the pooled sample was quantified spectrofluorimetrically using SYBR Green I (27) and standard solutions of E. coli DNA (Sigma).

dDNA media – The source of DNA was sodium salt, double-stranded DNA derived from salmon testes (Sigma, D-1626). From this source, High Molecular Weight (HMW) and Low Molecular Weight (LMW) dDNA media were created. For HMW-dDNA, 1 g of DNA was dissolved into 100 mL of distilled water. For LMW-dDNA, 1 g of DNA was dissolved into 100 mL of distilled water with 7000 units of DNAse I (Roche 104 132) in DNAse buffer (200 µM Tris pH 8.3, 500 µM KCl, 10 µM MnCl₂) at 37°C for 3 hours while continuously stirring. The DNAse reaction was stopped by heating at 70°C for 20 min. Deactivated DNAse (100°C for 1 hr) and DNAse buffer were added to the HMW-dDNA to control for the compounds that were used in making 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 while LMW-DNA was ≤ 250 bp (data not shown).
HMW- or LMW-dDNA was then added to sterile artificial seawater media at a final concentration of 1 g DNA/L. The seawater media contained 30 g/L of sea salts (Sigma 59883), 10 mM HEPES buffer, and 1 mL of a trace element stock. The trace element stock contained the following dissolved in 1 L of distilled H$_2$O: 5.2 g EDTA, 2.1 g FeSO$_4$, 30 mg H$_3$BO$_3$, 100 mg MnCl$_2$·4H$_2$O, 190 mg CoCl$_2$·6H$_2$O, 1 g NiCl$_2$·6H$_2$O, 2 mg CuCl$_2$·2H$_2$O, 144 mg ZnSO$_4$·7H$_2$O, 36 mg Na$_2$MoO$_4$·2H$_2$O, 6 mg Na$_2$SeO$_3$·5H$_2$O, and Na$_2$WO$_4$·2H$_2$O. Although HEPES is a buffer that contains organic carbon and organic nitrogen, control experiments demonstrated that *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 media prior to autoclaving. The washing process consisted of rinsing the agar seven times with distilled water, one time with 70% ethanol, and one time with acetone, followed by aeration at 40°C for three days. Control media consisted of all the above ingredients without DNA.

**Enrichments**—Dilutions of Eel Pond sample (100 µL of $10^0 - 10^{-4}$) 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 d, a subset of replicate plates was used for plate wash PCR (see below). After 10 d, bacterial abundance was estimated by calculating the total number of colony forming units (CFUs) for a set of replicate plates from each of the three dDNA treatments. One-way ANOVA and Tukey’s HSD were used to test for differences in CFU’s 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 the Ultraclean Fecal DNA isolation kit (MoBio Laboratories). 16S rRNA genes were amplified using bacterial universal primers (8f and 1492r) and the resulting PCR products were cloned using the Invitrogen TOPO TA\textsuperscript{®} cloning kit. A total of 106 clones were picked for sequencing (40 LMW and 66 HMW). Purified plasmids were utilized as templates for partial sequence determinations using the 16S rRNA gene bacterial primer 531R (5'-TAC CGC GGC TGC TGG CAC-3') with the BigDye Termination kit version 3.0 (Applied Biosystems). Completed reactions were run on a 96-capillary 3730xl DNA analyzer (Applied Biosystems). Sequences were aligned using the ARB phylogenetic software package (25). Sequenced 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 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 their original dDNA media. Individual colonies were lysed by boiling in 20 µL of 0.05% Triton X-100 for 5 min. One microliter of the resulting supernatant was used as a template in a 25µl PCR reaction. The 37 PCR products were screened by agarose gel electrophoresis and sequenced (see above) after cleaning with the ExoSAP-IT\textsuperscript{®} kit (USB Corporation). Phylogenetic identification of the sequences was determined by finding the most closely related representatives in the BLAST database (www.ncbi.nlm.nih.gov/BLAST). A total of 37 isolates were identified (18 from HMW-dDNA and 19 from LMW-dDNA).
Growth curves on dDNA – Nineteen of the phylogenetically identified isolates from LMW-dDNA plates were used in a set of growth curve experiments in liquid dDNA media. The liquid media experiments were performed to characterize bacterial growth on different sized dDNA. The composition of the liquid medium was the same as described above (see dDNA media) without the addition of agar. For each isolate, there were a total of six test tubes (15 mL); three replicates contained LMW-dDNA and three replicates contained HMW-dDNA. The tubes were inoculated with a homogenous suspension of cells (100 µL of a 0.9% NaCl suspension) and incubated in an upright shaker at 30°C. Growth was monitored as the change in absorbance at 600 nm (OD 600) over time (110 h). The effect of dDNA type (HMW vs. LMW) 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 RM-ANOVA model indicates that growth increased over the duration of the experiment irrespective of dDNA treatment. A significant effect of dDNA indicates that growth was different between LMW- and HMW-dDNA irrespective of time. A significant interaction (time x dDNA) indicates that changes in growth over the duration 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. An assay was developed to evaluate the ability of different bacterial isolates to degrade HMW-dDNA. The assay is similar to the single radial enzyme diffusion method (SRED), which uses a nucleic acid stain (e.g., SYBR Green or ethidium bromide) to quantify the clearing of DNA on agarose gel plates due to DNAse activity (42). dDNA degradation was assayed for a total of 64 isolates (32 LMW-dDNA isolates and 32 HMW-dDNA isolates), 19 and 18 of which had positive phylogenetic identifications,
respectively. For each isolate, a small amount of cell biomass was aseptically transferred into
250 μL of 0.9% NaCl after lightly touching the tip of a toothpick to a single colony. In
duplicate, 50 μL of a cell suspension was spotted onto the middle of 100 mm diameter Petri
dishes containing HMW-dDNA agar media (see dDNA media). The Petri dishes were incubated
at 30°C for 10 d and were then soaked in a 1X TBE bath with ethidium bromide (100 μg L⁻¹) for
2 min. The soaked plates were rinsed in a 1X TBE bath for 1 min before taking images under
UV light with a transilluminator adapted with image analysis software. Positive controls
consisted of 50 μL (7000 units) additions of DNase I to a Petri dish containing HMW-dDNA
agar. The area of DNA-degradation was quantified relative to the area of the entire Petri dish
using AxioVision software (version 4.4). Percent data were then converted to rates of dDNA
degradation assuming that zones of clearing were completely depleted of HMW-DNA. 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 – Salinity, temperature, and conductivity were uniform throughout the water column in Eel Pond. In contrast, dissolved oxygen concentrations decreased with depth from 7.3 to 3.6 mg L$^{-1}$. The average abundance of DAPI-stained bacteria in the pooled water sample was 1.3 (± 0.71 SE) x 10$^7$ cell mL$^{-1}$. The mean dDNA concentration in the pooled water sample was 26 (± 0.3 SE) µg L$^{-1}$.

Diversity of dDNA consuming bacteria – Of the 106 sequences generated from the plate wash PCR, a total of 32 taxa were identified using a 99% sequence similarity cutoff. Taxon richness was comparable between the LMW- and HMW-dDNA clone libraries (18 and 21, respectively). However, only seven of the 32 taxonomic groups (22%) contained sequences from both treatments; the remaining 25 taxonomic groups (78%) contained either LMW or HMW sequences. Fisher’s exact test indicated that the composition of the clone libraries were significantly different from one another ($P = 0.0001$). Most (88%) of the taxa belonged to the $\gamma$-proteobacteria and were represented by *Vibrio* spp., *Alteromonas* spp., and *Pseudoalteromonas* spp. Two sequences belonged to the $\alpha$-proteobacteria; one of these 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 was closely related to a *Flexibacter* sp. (95% similarity) and the other was most closely related to *Microscilla arenaria* (94%). Some sequences formed monophyletic groups with respect to their dDNA source. For example, *Vibrio* sequences were only recovered on LMW-dDNA. In addition, there were three clades of HMW *Pseudoalteromonas* and a single clade of...
LMW *Alteromonas*. The largest clade was the “mixed” *Alteromonas* group, which comprised 17 LMW sequences and 29 HMW sequences (Fig. 1).

**Bacterial abundance on dDNA** – The average number of CFUs was higher on LMW-dDNA than HMW-dDNA agar plates (5.2 ± 0.32 X 10^5 cell mL^-1 vs. 2.1 ± 0.43 X 10^5 cell mL^-1, ANOVA, *P* < 0.0001; Fig. 2). Control plates had significantly fewer CFUs (1.5 ± 0.99 X 10^4 cell mL^-1) than both the LMW-dDNA and HMW-dDNA agar plates (ANOVA, *P* < 0.0001).

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

**dDNA degradation** – Degradation of dDNA by marine bacteria was readily apparent with the ethidium bromide assay. For the negative control, dDNA fluoresced evenly across the agar surface (Fig. 4a), whereas distinct clearing 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 4d). Bacteria originally isolated from HMW enrichments degraded twice as much HMW-dDNA as bacteria originally isolated from LMW enrichments (14 ± 2.0 vs. 7 ± 2.0 mg L^-1 d^-1, *t*-test, *P* = 0.015, Fig. 5). It was determined whether this relationship held for different phylogenetic groups of bacteria.
that occurred in both of the dDNA treatments. Rates of HMW-dDNA degradation were not
different for *Pseudoalteromonas* spp. (*t*-test, *P* = 0.5), but *Alteromonas* spp. isolated from
HMW-dDNA enrichments degraded 3 times more DNA than *Alteromonas* spp. isolated from
LMW-dDNA enrichments (15 ± 3.0 vs. 5 ± 3.0 mg L⁻¹ d⁻¹, *t*-test, *P* = 0.04).

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DISCUSSION

Dissolved DNA (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 the physiology of dDNA consuming bacteria. Some, but not all, of the marine microbes isolated in this study had the ability to take up and efficiently use dDNA as a primary source of energy and nutrition.

Bacterial isolates typically experienced lower growth on HMW-dDNA than on LMW-dDNA, especially if they were originally isolated from LMW enrichments. This reduction in bacterial growth may reflect energetic costs associated with nuclease production, or in some cases, an inability to produce nucleases altogether (2). Results from this study suggest dDNA size can influence microbial diversity, but 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 successional sequence of microbial assemblages with different metabolic capabilities.

Diversity of dDNA consuming bacteria – A relatively diverse assemblage of marine bacteria was cultivated in this study, especially when considering that a single molecule was used as the primary source of carbon, nitrogen, and phosphorus. Bacteroidetes and α-proteobacteria comprised 12% of the taxa in the clone libraries, while the remainder were representative of the γ-proteobacteria. This latter group was dominated by Alteromonas and Pseudoaltermonas, which are well-known for their ability to degrade high molecular weight compounds via extracellular enzyme production (9, 28).
Culture-independent surveys suggest the abundance of γ-proteobacteria, however, is generally low compared to other groups of bacteria in marine ecosystems (16). Despite being relatively rare, the γ-proteobacteria are 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 (e.g., upwelling, eddies, or bloom dynamics) and small-scale (e.g., sloppy grazing and viral lysis) processes. Some studies suggest 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 only provide 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 bacteria represented in the clone libraries. Initially, it was predicted that bacteria which grew on HMW-dDNA would be a subset of the LMW-dDNA bacterial assemblage. 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, taxon richness was comparable between the two treatments. 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 *Pseudoaltermonas* 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 influences 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 compositions found in the different DOM size fractions. For example, the response of bacterial community composition to DOM additions in the Delaware Bay was dependent on the identity of the HMW molecule under investigation (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.

**Bacteria growth affected by dDNA size** – Changes in microbial composition corresponded with trends in the abundance and growth of bacteria on LMW- and HMW-dDNA. First, bacterial abundance was approximately 2.5 times greater on LMW versus HMW-dDNA plates (Fig. 2). Second, half of the strains isolated from LMW enrichments experienced reduced growth when inoculated into HMW-dDNA liquid media (Table 1; 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 high molecular weight molecules (6).
Results from the growth curve experiments also provide insight into a potential mechanism that may have contributed to observed differences in microbial composition. Specifically, bacteria isolated from the LMW enrichments generally performed better when grown in LMW liquid media (Table 1; Fig. 3) and thus may have out-competed HMW strains under these conditions.

DNA degradation varies among marine bacteria – Results from 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 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 enrichments degraded HMW-dDNA two times faster than bacteria isolated from the LMW enrichments (Fig. 5).

The ethidium bromide assay, which has not been used in the past for assessing dDNA degradation by marine bacteria, is similar to assays used for measuring nuclease activity (42, 12). Therefore, it is reasonable to assume that the observed DNA degradation in this study reflects some integrated features of bacterial nuclease production, which might include variation in specific activity, production rates, or diffusive properties of different enzymes. Moreover, results from 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 from 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 enrichment conditions. In contrast, dDNA degradation rates were three times greater for *Alteromonas* spp. isolated from HMW enrichments than *Alteromonas* spp.
isolated from LMW enrichments suggesting that observed “microdiversity” may equate to 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).

There are currently two models used to explain bacterial uptake of dDNA for nutritional purposes. First, 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 has not been well-demonstrated 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 lend support to 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 topic suggest that many species of bacteria isolated from marine environments, including Pseudomonas, Flavobacterium, Vibrio, and Acinetobacter, 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 4d). This pattern of dDNA uptake is more consistent with extracellular enzyme degradation (26) than competency, which requires
binding of DNA with the bacterial cell (5). Thus, results from the current study lend support to the model of extracellular dDNA degradation followed by uptake of 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 from this study indicate that populations of co-occurring marine bacteria were specialized at using either LMW- or HMW-dDNA. On average, bacteria isolated from LMW-dDNA enrichments had higher growth on LMW-dDNA than on HMW-dDNA (Fig 3, 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 sized dDNA suggests a trade-off, which could help maintain microbial diversity (21) and influence ecosystem processes. For example, resource specialization by distinct groups of bacteria within 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 those found in a recent simulation model that explores the interactions between microbial populations receiving HMW organic substrates (1). In the model there are two types of bacteria: those that are directly responsible for HMW substrate degradation via extracellular enzymes (“producers”) and those that take advantage of the extracellular enzymes generated by producers (“cheaters”). Simulations revealed that cheaters experienced a selective advantage when enzyme cost increased for producers. In contrast, producers were favored in heterogeneous environments or when enzymes remained in close proximity to the producer’s cell surface. Ultimately, the model predicted that producers and cheater would coexist at intermediate levels of enzyme cost and
intermediate levels of enzyme diffusivity. Results from this experimental study suggest that the dDNA system may be ideal for testing theoretical predictions about extracellular enzyme activity, microbial interactions, and DOM dynamics.

Prior studies have documented rapid uptake of dDNA by aquatic microbial communities suggesting it 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 successional sequence of microbial assemblages with different metabolic capabilities.
ACKNOWLEDGMENTS

I acknowledge an unnamed colleague for his help during many stages of this project; T.M. Schmidt and W.W. Metcalf for discussions on the DNA degradation assay; results from a preliminary study by C.P. Lostroh; J.A. Breznak for advice on media preparation, H.E. Reed for assistance with phylogenetics; D.E. Hunt for *Vibrio* tester strains; N.C. Caiazza, A.M. Wier, K. Milferstedt, H.E. Reed, and J.M. Palange for critical comments on an earlier version of the manuscript. Financial support was provided from the Nathan Jenks Biology Prize, the Milton L. Shifman Endowed Scholarship, the Albert Cass Fellowship, and the Microbial Diversity summer course at the Marine Biological Laboratory, Woods Hole, MA. This is Kellogg Biological Station contribution number 1288.
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Table 1. Results from Repeated Measures Analysis of Variance (RM-ANOVA) for bacterial growth on dDNA. Nineteen strains from the LMW enrichments were isolated and phylogenetically identified via 16S rRNA gene sequencing. These isolates were then grown in liquid media containing either LMW- or HMW-dDNA for 110 h. Bacterial growth was measured as absorbance at 600 nm (OD 600). Significant \( P \)-values are reported for highest order effect (i.e., DNA x time), followed by significant main effects (DNA or time); ns = not significant.
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FIGURE LEGENDS

Figure 1. Unrooted phylogenetic tree for 16S rRNA gene sequences collected from bacterial assemblages that developed on LMW- and HMW-dDNA. Values in parentheses represent the number of clones found in a given clade.

Figure 2. Colony forming units (CFUs) from enrichments containing no added DNA (i.e., control), HMW-dDNA, and LMW-dDNA. Data equal mean ± SEM. Probability ($P$) value refers to the result of a one-way ANOVA.

Figure 3. Representative growth curves (OD 600 nm) 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 media. Data equal mean ± 1 SEM. Probability ($P$) values refer to results from repeated measures ANOVA. Note different OD 600 scales.

Figure 4. Ethidium bromide assay used for documenting 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. Images in C and D were taken after ethidium bromide staining of HMW-dDNA plates, which had incubated for 10d following the spotting of a cell suspension at the center of the plate.
Figure 5. Rates of HMW-dDNA degradation from the ethidium bromide assay for strains originally isolated from LMW- and HMW-dDNA enrichments. Probability ($P$) value refers to the result of a $t$-test.
HMW-dDNA degradation (mg L⁻¹ h⁻¹)

\[ P = 0.015 \]

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\( P = 0.015 \)