How many bacterioplankton in oxic surface waters can grow anaerobically? A comparison across nutrient gradients

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Abstract

A large fraction of culturable marine bacterioplankton from seven sites in the Mid-Atlantic Bight grew as facultative anaerobes on a variety of carbon substrates. This fraction varied systematically with nutrient levels. On average colony-forming units (cfu) counts under anaerobic conditions were 52% of cfu counts under aerobic conditions in a nutrient rich coastal environment (Woods Hole Harbor), in contrast to 20% from the oligotrophic Sargasso Sea. On glucose alone the difference was 92% for coastal bacterioplankton versus 28% for Sargasso bacterioplankton. These results suggest that anoxic microhabitats in the oxic ocean are more common than previously thought. They also indicate a potential selective advantage for bacteria in oxic surface waters capable of facultatively anaerobic growth. Open ocean bacterioplankton had similar cfu counts on several substrate types, while Woods Hole bacterioplankton had five times as many cfu on glucose as on any other substrate. Sequences obtained from individual isolates were predominantly gamma-Proteobacteria (91%). Isolates from the Sargasso Sea were predominantly related to Psychrobacter marinicola-related phylotypes were dominant in Sargasso Sea isolates, while various Vibrio spp. were dominant in shelf, slope, and coastal isolates.

Introduction

The surface waters of the global ocean are oxic and generally nutrient-limited. Given this environment, one might expect that the vast majority of pelagic heterotrophic bacteria are strict aerobes. This naïve assumption is brought into question by a closer consideration of the microenvironments actually occupied by pelagic bacteria. Organic matter aggregates, known as “marine snow,” are hotspots for bacterial activity in the open ocean ((14) and references therein). These aggregates typically show higher concentrations of bacteria than the surrounding water, higher concentrations of inorganic
nutrients and dissolved organic matter, and higher activity of proteolytic and hydrolytic exoenzymes (2). Several studies from different oceanic regimes have shown that the attached bacterial community is significantly enriched in Cytophaga/Flavobacteria and gamma-Proteobacteria relative to the free-living community, which is typically dominated by alpha-Proteobacteria (1, 4, 14). Bacteria on aggregates tend to differ physiologically from the free-living community: they have higher cell specific uptake rates of free amino acids and monosaccharides, are generally larger, and grow in microcolonies or filamentous forms (14). The aggregates may also be anoxic in the interior, allowing anaerobic bacterial growth via fermentation, although the availability of such habitats to pelagic bacteria is largely unknown.

One can get indirectly at the question of whether these anoxic microzones are significant in aerobic water columns, simply by asking what proportion of bacteria in pelagic assemblages are capable of facultatively anaerobic growth. The bacteria which colonize aggregates must spend a large part of their lifespan in the aerobic water column. If they maintain the genomic capacity for fermentative growth, it follows that there is a selective advantage to doing so, which in turn implies that anoxic microhabitats must occur at some frequency. Indeed, methanogens (which are strict anaerobes) have been isolated from oceanic particulate matter (14).

This question has never been addressed for natural pelagic assemblages. Its potential significance is suggested by two observations. First, bacterial biomass on aggregates can exceed 30% of total bacterial biomass in eutrophic and estuarine systems (14). Second, Riemann & Azam (12) observed that 63% of a collection of marine isolates capable of degrading N-acetyl-D-glucosamine (a component of chitin and peptidoglycan) had the capacity to grow as facultative anaerobes using fermentation of mannitol or glucose. This surprisingly high number suggests that a substantial fraction of bacteria in pelagic marine assemblages could have the capacity for anaerobic growth.

This study is a preliminary test of the hypothesis that the fraction of pelagic bacteria capable of facultatively anaerobic growth is higher in nutrient-rich waters, where aggregates and other anoxic microenvironments are more likely to be present. I also asked whether the percentage of bacteria which appear first on aerobic incubation plates and can grow as anaerobes is higher than the percentage of late-appearing bacteria on
aerobic plates which can be grown as anaerobes. This follows from the idea that bacteria which can grow as facultative anaerobes are also those bacteria most likely to respond rapidly to changes in environmental nutrient concentration. Incubations of soil bacteria have found a division between fast-growing, early appearing bacteria with high rrn copy number and slow-growing, late-appearing bacteria with low rrn copy number (6). If this pattern holds among oceanic bacterioplankton, I would expect a systematic difference in the spectrum of growth conditions between early and late isolates. There is some support for this growth pattern among common coastal heterotrophs. For example, *Vibrio* spp. are typical early isolates from coastal waters on high nutrient plates (pers. obs.), have the capacity for facultatively anaerobic growth, and as a group have a high average rrn copy number (9.2, (7)).

I obtained seawater samples taken in a transect from the oligotrophic Sargasso Sea to nutrient-rich Woods Hole Harbor. These were plated on a series of solid media with different carbon substrates, and identical sets were incubated in aerobic and anaerobic environments. Substrates were chosen to represent breakdown products of cell lysis and dominant biopolymers in marine snow aggregates (14), particularly various sugars, amino acids, purines, and cell wall components. The comparison of samples from high and low nutrient seawater is crucial to a proper test of the hypothesis. Typically only 0.1-1% of bacteria in natural assemblages can grow on laboratory media, which excludes bacteria requiring low nutrient levels (such as the ubiquitous SAR11 clade). If one assumes that this culturability bias is roughly comparable between the two habitats, a comparison between the two should control for it. Here I report the results of a preliminary study conducted in July 2004.

**Materials and Methods**

**Environmental samples**

Samples were kindly collected in late June and early July 2004 by Patrick Rowe, shipboard technician on the WHOI R/V *Oceanus*, on the vessel’s return from Bermuda (cruise OC 404-1, Dennis McGillicuddy, Chief Scientist). Samples were collected in
triplicate from surface waters by dragging 50 mL conical tubes over the side of the vessel while it was underway. Sample sites were selected based on identification of different water masses from a current (6/23/04) satellite SST map of the area (Fig. 8). Sample locations are shown on Figure 8. Samples were collected at 6 locations from the Oceanus (Table 1), as well as on a small motorboat from Woods Hole Harbor. Oceanus samples were stored on board ship for several days at 4°C during transit.

Stations O1 (BATS) and O2 (Sargasso) should be similar in terms of nutrient and temperature profiles, although I did not receive ship data to confirm this. Both are located in nutrient limited oligotrophic waters in the Sargasso. At BATS, DOC is typically constant in the summer at around 68 μM, in situ temperatures are 27-28°C, and inorganic N and P are below detection limits (3). Station O3 was located in the center of an anticyclone, which is a mesoscale eddy marked by warm-water anomalies relative to the surrounding ocean. The SST map (Fig. 8) shows that station O4 is located in the middle of the Gulf Stream, which is noticeably warmer than the Sargasso. Stations 5 and 6 are located in cooler waters.

<table>
<thead>
<tr>
<th>Station ID</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31.67</td>
<td>64.17</td>
<td>Bermuda Atlantic Time Series (BATS)</td>
</tr>
<tr>
<td>2</td>
<td>33.00</td>
<td>65.50</td>
<td>Sargasso NE of Bermuda</td>
</tr>
<tr>
<td>3</td>
<td>35.00</td>
<td>67.00</td>
<td>Sargasso Anticyclone</td>
</tr>
<tr>
<td>4</td>
<td>37.00</td>
<td>68.50</td>
<td>Gulf Stream</td>
</tr>
<tr>
<td>5</td>
<td>39.00</td>
<td>69.50</td>
<td>Slope Water</td>
</tr>
<tr>
<td>6</td>
<td>41.00</td>
<td>71.00</td>
<td>Shelf Water</td>
</tr>
</tbody>
</table>

Table 1: Sample locations from R/V Oceanus

Enumeration of cells using DAPI

I fixed 4 mL of water from each of the Oceanus samples with an equal volume of 100% EtOH for a few hours, then filtered the whole volume onto a 47 mm white 0.2 μm filter and rinsed with a 2% NaCl solution. I cut a filter section and incubated it for 3 min with 1 μg/ml DAPI, then rinsed in 70% EtOH and water prior to mounting on a glass slide with Citiflour. Cells were counted at 100x magnification on a Zeiss Axiophot fluorescence microscope.

Growth experiments
Media preparation

I prepared a base media for aerobic and anaerobic plating of 1.5% agar in 80% ASW, with the addition of 1 mL/L trace metals and 1 mL/L vitamin solution prepared according to recipes distributed in the course. I also added 0.01% yeast extract to all media as a basal nutrient source. The media was adjusted to pH 7.3 with 1M NaHCO₃. I prepared plates with the following additional carbon substrates: glucose, cellobiose, ribose, chitin, adenine, and casein. Glucose, cellobiose, and ribose were added from 0.5 M sterile stock solutions after autoclaving of the base media to a final concentration of 5 mM. Phenol red was added to the glucose medium from a 5 g/L stock to achieve a final concentration of 0.01 g/L. Phenol red is a pH indicator which is pink at neutral pH and yellow at acid pH, and was used to identify colonies carrying out fermentation with acidic end products. Casein was added to a final concentration of 0.05%. Adenine was prepared by dissolving 1.5 g in 80 mL boiling water. Addition of 1 mL 5N NaOH was necessary to get the adenine into solution. The pH was then adjusted to 7.2 with 6N HCl and added to the medium to reach a final concentration of 5 mM. Chitin was prepared as an overlay medium as described in (10). Briefly, I prepared a slurry with 10 g unbleached chitin in 200 mL 100% HCl. The slurry sat at room temperature 30 min until it turned dark brown/black. It was then mixed with 500 mL ice cold MilliQ water and washed extensively on a Buchner funnel lined with a Whatman GF/F filter. The resulting slurry was placed in dialysis tubing and dialyzed against tap water overnight. I added DI water to the suspension, adjusted the pH to 7.2 with concentrated KOH, and determined the final volume. I then added the slurry to the base agar medium to a concentration of 0.5%, autoclaved, and poured as an overlay over cooled base medium plates.

Plating and incubation

The experiment was designed to incubate undiluted and concentrated samples of seawater on identical sets of plates under aerobic and anaerobic conditions. I plated 100 μl of environmental seawater directly onto two sets of plates, containing all six substrates above plus a control containing only 0.01% yeast extract, trace metals, and vitamins. I
also concentrated each sample 10-fold by filtering 50 ml onto a 0.2 μm pore size filter mounted in a Swinnex cartridge, and resuspending in 5 ml filtrate. I plated 100 μl of this concentrated filtrate on another two sets of plates. All plating was done aerobically on the benchtop.

Identical sets of plates were incubated at room temperature on the benchtop and under a N₂ atmosphere in modified Difco and BBL gas jars. Sealed jars were prepared by repeated vacuuming with a pump and flushing with pure N₂ both before and after addition of the plates. Methylene blue strips were added to each jar to determine anaerobiosis. Jars were flushed with N₂ each day to maintain anaerobiosis.

**Enumeration**

I counted all colonies visible to the naked eye at timepoints of 4, 7, and 11 or 12 days for all treatments. I picked a subset of colonies appearing at each timepoint for phylogenetic identification and noted typical “early” and “late” colony phenotypes.

**Restreaks**

I streaked out selected “early” (colonies appearing four days after plating) and “late” (colonies appearing seven days after plating) from the aerobic plates onto identical media and placed these plates in a gas jar for anaerobic incubation. Growth was checked after four days.

**Colony PCR and sequencing**

Picked colonies were placed into 20 μl 0.05% Tween in a 96-well PCR plate, frozen, then lysed at 100°C for 5 min in a MJ Research thermocycler. PCR targeting the 16s rRNA gene was performed on the lysed colonies using the universal eubacterial primers 8F and 1492R with the following cycle parameters: 95°C for 5 min, followed by 25 cycles of 95°C for 30s, 55°C for 30s, 72°C for 1 min, and one 5 min extension at 72°C. Products were sequenced directly on an ABI capillary sequencer at the Bay Paul Center, Marine Biological Laboratory. Sequences were aligned in ARB (8) and added
with neighbor-joining techniques to trees constructed with longer sequences. Bootstrap analysis was not done because the sequences were too short (<500 bp).

Results/Discussion

The culturable fraction

Total cell numbers increased an order of magnitude in the transect from BATS to Woods Hole (Fig. 1). Culturable cells on the media used ranged from tens per ml at BATS to hundreds per ml in shelf water (station O6) and thousands per ml in Woods Hole Harbor, but were in all cases about 0.01% of the DAPI cell count. The number of colony forming units (cfu) per mL appeared to correlate with the temperature and/or chlorophyll concentration at each sampling site. Stations O1-O3 are in zones of higher SST than stations O5, O6, and WH, while the reverse is true for chlorophyll concentration (SeaWIFS, http://seawifs.gsfc.nasa.gov). High chlorophyll concentration generally indicates a greater availability of labile carbon substrates.

Within the “oligotrophic set” (O1, O2, and O3), cfu/mL counts were elevated at station O3. Station O3 is located in an anticyclone, which shows elevated SST compared to surrounding waters (Fig. 8), and also shows higher cfu counts than station O2 north of Bermuda or station O5 in Slope Water. This could indicate a positive effect of temperature on bacterial growth rate and/or a greater abundance of labile substrates than surrounding waters.

Substrate utilization patterns

An unexpected pattern emerged in the number and evenness of substrates used at each site. Samples from BATS, shelf water, and slope water all showed a greater diversity of substrate utilization than samples from Woods Hole harbor. Similar numbers of cfu/ml formed for three different substrates at stations O1, O3, O5, and O6, whereas Woods Hole harbor had five times as many cfu/ml on glucose as on the next substrate (Table 2). This may indicate that bacterioplankton living in low-nutrient environments are better adapted to use multiple, potentially transitory substrates than bacterioplankton...
in high nutrient environments. This explanation is confounded somewhat by the dominance of casein utilizers at station O2, which is difficult to account for without further information on ambient nutrient levels.

The optimal substrates for growth varied between sample stations (Table 2). In general the two hexose sugars (glucose and cellobiose) along with the ribose were preferred substrates. The multiple pathways for glucose utilization in bacteria are well-characterized, and it is the dominant monosaccharide in some oligotrophic waters (11). Cellobiose is simply a repeating polymer of glucose. Chitinolytic bacteria were found only in cooler waters (stations O5, O6, and WH). Extensive clearing of the chitin overlay was visible in both aerobic and anaerobic incubations from these stations. The abundance of chitinolytic colonies at station O6 was surprising, and may indicate a transitory peak in chitin abundance at that site. Very little anaerobic growth was seen on the adenine plates, which is expected as the only bacteria known to ferment adenine to yield energy for growth are strictly anaerobic Clostridia (5). Aerobic growth on adenine was also low in comparison with the other added substrates. Except at station O6, it was comparable to growth on the base yeast extract medium, probably indicating that organisms were not using adenine as an energy-yielding substrate for growth. Sequence related to a coastal Vibrio isolate (9) was obtained from a colony growing on adenine plates at station O6. As the substrate utilization patterns of the published isolate are unknown, it is an open question whether Vibrio spp. can grow using adenine as an energy source. Casein, a mixture of hydrolyzed proteins, was by far the dominant substrate utilized at station O2. Growth on other added substrates did not exceed growth on basal medium (with the unexplained exception of the anaerobic day 12 cellobiose count). The dominant phylotypes at station O2 were all related to Halomonas spp., in clear contrast to stations O1 and O3, both dominated by Psychrobacter marinicola.

Colony morphologies also differed broadly between the lowest and highest nutrient sites, with the Sargasso Sea samples dominated by small colonies, even after 12 days, and the coastal samples dominated by large colonies and spreaders. This result could suggest that bacterioplankton adapted to high nutrient environments are capable of more rapid growth in response to nutrient addition.
Table 2: Optimal growth substrates at different stations from aerobic plates, day 12. Numbers are culturable cells on a given C substrate per mL seawater. *Casein colonies were too numerous to count individually; plate total is estimated. Seawater from station O4 did not show growth on any substrates except casein.

<table>
<thead>
<tr>
<th>Substrate/Station</th>
<th>Ribose</th>
<th>Glucose</th>
<th>Cellulose</th>
<th>Chitin</th>
<th>Casein</th>
<th>Adenine</th>
<th>Yeast extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>O1</td>
<td>50</td>
<td>47</td>
<td>44</td>
<td>0</td>
<td>9</td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td>O2</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>66</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>O3</td>
<td>175</td>
<td>180</td>
<td>174</td>
<td>93</td>
<td>146</td>
<td>107</td>
<td>-</td>
</tr>
<tr>
<td>O4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>25</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>O5</td>
<td>30</td>
<td>70</td>
<td>11</td>
<td>77</td>
<td>75</td>
<td>44</td>
<td>-</td>
</tr>
<tr>
<td>O6</td>
<td>212</td>
<td>484</td>
<td>215</td>
<td>568</td>
<td>500*</td>
<td>191</td>
<td>0</td>
</tr>
<tr>
<td>Woods Hole</td>
<td>210</td>
<td>1090</td>
<td>50</td>
<td>110</td>
<td>130</td>
<td>90</td>
<td>50</td>
</tr>
</tbody>
</table>

Anaerobic vs. aerobic incubations

In most cases cumulative colony forming curves showed an increase in cfu from day 4 to 12 (Figs. 2-6). The pattern of growth curves was similar between aerobic and anaerobic incubations, although anaerobic plates typically showed a much lower cfu count (Table 3). Surprisingly, some anaerobic plates in the lowest nutrient stations (O1 and O2, both in the Sargasso Sea) showed a higher cfu count than identical aerobic plates. It is unclear whether this is a meaningful result or simply biased by the very low number of cfu on these plates (<10 in all cases). In most cases plates from the tenfold concentrated inoculum gave much more consistent counts, with the exception of Woods Hole harbor, where the high number of cfu made the undiluted sample easiest to count.

The rate of appearance of colonies did not differ systematically across the transect, using (# colonies at 4 days/# colonies at 12 days) as a proxy for rate. However, a plot of this proxy for aerobic vs. anaerobic incubations for the same sample showed a curious, nearly bimodal distribution (Fig. 7). Treatments tended to show either small (>0.7, "fast") or large (<0.3, "slow") differences or between early and late colony numbers on anaerobic plates. There does not appear to be any correlation with sample site, but glucose plates are overrepresented five-fold in the "fast" category.

The most relevant result for the initial hypothesis is the fraction of anaerobic vs. aerobic colonies at each sample site. The interpretation of these results (Table 3) is not straightforward, because the Bermuda-Woods Hole transect was not a steady progression from low to high nutrient levels. Stations O3 and O6 in particular break the trend in informative ways. For station O3, increased temperature in the anticyclone appears to
have lead to slightly increased total cell numbers, cfu per mL, and facultative anaerobic growth as compared with the flanking stations. Slope water (station O6) had cfu counts of order 10^2 per mL, but low anaerobic growth on every substrate except ribose.

Broadly, however, these results support the initial hypothesis: on a variety of labile substrates, the average percentage of bacteria capable of facultatively anaerobic growth is higher in a nutrient-rich coastal harbor (WH, 52%) than in the nutrient-poor Sargasso Sea (O1, 20%). The difference is larger when only glucose is considered: 28% of cfu grew as facultative anaerobes at BATS, while 92% of cfu grew as facultative anaerobes in Woods Hole Harbor. This result is broadly consistent with the observation that a majority of coastal marine heterotrophic isolates in a small culture collection could grow as facultative anaerobes (12). The gas jars used are not impermeable to oxygen so it is unlikely that any strict anaerobes were isolated in this study. It would be necessary to use an additional reductant in the media and/or incubate plates in an anaerobic chamber to identify strict anaerobes.

<table>
<thead>
<tr>
<th>Glucose</th>
<th>Ribose</th>
<th>Cellubiose</th>
<th>Casein</th>
<th>Chitin</th>
<th>Adenine</th>
<th>YE</th>
<th>Average (all)</th>
<th>Average (&lt;1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O1</td>
<td>0.28</td>
<td>0.14</td>
<td>0.11</td>
<td>1.2</td>
<td>5</td>
<td>(no growth)</td>
<td>0.28</td>
<td>1.2</td>
</tr>
<tr>
<td>O2</td>
<td>1.25</td>
<td>1</td>
<td>11.7</td>
<td>0.11</td>
<td>(no growth)</td>
<td>3</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>O3</td>
<td>0.31</td>
<td>0.34</td>
<td>0.77</td>
<td>0.36</td>
<td>0.89</td>
<td>0.10</td>
<td>n/a</td>
<td>0.46</td>
</tr>
<tr>
<td>O5</td>
<td>0.31</td>
<td>0.2</td>
<td>1.4</td>
<td>0.28</td>
<td>0.47</td>
<td>(no ana growth)</td>
<td>0.53</td>
<td>0.32</td>
</tr>
<tr>
<td>O6</td>
<td>0.06</td>
<td>0.32</td>
<td>0.06</td>
<td>0.03</td>
<td>0.18</td>
<td>0.005</td>
<td>n/a</td>
<td>0.11</td>
</tr>
<tr>
<td>WH</td>
<td>0.92</td>
<td>0.62</td>
<td>n/a</td>
<td>0.54</td>
<td>0.27</td>
<td>0.56</td>
<td>0.2</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Table 3: Cumulative ana colonies/cumulative aer colonies on day 12. Station O4 is omitted due to lack of growth on any substrate except casein under aerobic conditions.

Restreaks of early and late-appearing colonies on anaerobic plates

I predicted that colonies appearing early on aerobic plates would be more likely to grow as facultative anaerobes than colonies appearing later on aerobic plates. The reverse result was obtained: all “late” isolates could grow under anaerobic conditions, while early isolate growth ranged from 44-100% (Table 4). This result may be due to
inadequate anaerobiosis in the gas jar used for incubation of late isolates. Unfortunately there was not time to repeat the experiment to test this. It is also possible that colonies appearing at day 7 are not “late” enough to show different growth patterns than colonies appearing at day 4.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>% growth of early isolates</th>
<th>% growth of late isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>Ribose</td>
<td>44</td>
<td>100</td>
</tr>
<tr>
<td>Glucose</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Chitin</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>83</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 4: Percentage of early and late appearing aerobic colonies which grew under anaerobic conditions.

Sequence data

Several interesting patterns emerged from the 16s rRNA sequencing of selected early and late aerobic and anaerobic isolates (Fig. 9). The seventy sequences obtained were dominated by gamma Proteobacteria (91%), followed by Roseobacter sp. in the alpha Proteobacteria (4%), actinobacteria (3%) and low G+C Gram positive (1%). One phylotype was clearly dominant at each station, suggesting that the sequencing strategy used was successful in obtaining the most abundant culturable phylotype.

Different sets of culturable bacteria dominated in samples from warmer water (O1-O3) and cooler water (O5, O6, Woods Hole). Stations O1 and O3 were dominated by Psychrobacter marincola-related phylotypes and secondarily by Cobetia marina-related phylotypes, while Halomonas-related phylotypes were most abundant at station O2. The cold water set was dominated by various Vibrio spp. Roseobacter spp. were only observed in the two coastal stations (O6 and Woods Hole). A gradient in phylotype distribution is observable with Psychrobacter present in O1-O3 and Vibrio present in stations O3 through Woods Hole. The type species Psychrobacter marincola has a growth optimum at 25-28°C (13), the average temperature across wide swaths of the Sargasso Sea. Various Psychrobacter species are also capable of acid fermentation and utilization of a wide range of carbon substrates, which could also help explain their apparent dominance in the culturable fraction. Vibrio spp. are well known to be
extremely diverse metabolically, so their abundance in the coastal samples is unsurprising.

Two additional results from the clone library are of interest. The actinobacterium *Brachybacterium paraconglomeratum* LMG 19861 T was found growing aerobically on glucose in samples from Woods Hole, and anaerobically on chitin in samples from BATS. *B. paraconglomeratum* formed distinctive bright yellow, round colonies on both substrates and did not appear to be chitinolytic. *B. paraconglomeratum* thus appears to be widely distributed in the North Atlantic, although it has not been previously identified in this environment. A novel gamma-proteobacterial phylotype appeared on basal medium from Woods Hole Harbor. It was only 93% related by 16s rRNA to the closest cultivated relative, *Oceanoimonas smirnovii*, isolated from the Black Sea (GenBank, unpublished), suggesting that novel uncultivated bacteria can still be obtained by simple plate-based isolation methods.

**General conclusions**

This study characterized for the first time the percentage of culturable bacteria in anoxic marine habitats that have the capability to grow as facultative anaerobes on a range of carbon substrates. As hypothesized, this percentage was higher in high-nutrient coastal waters (52%) than in the oligotrophic Sargasso Sea (20%). These results strongly suggest a selective advantage for marine bacterioplankton to retain the genomic capacity for anaerobic growth. The dependence on nutrient levels also suggests that the magnitude of this advantage is determined by the availability of habitats for such growth.

Here the argument becomes largely speculative. We do not know the magnitude of each factor which might contribute to this putative selective advantage. It would be important to characterize: (1) the genomic cost of maintaining the capacity for anaerobic growth, (2) the probability that an individual bacterium will encounter anoxic microenvironments, correlated with (3) the spatial and temporal frequency of such microenvironments, and (4) the amount of time spent by an individual bacterium in the microenvironment. These are difficult parameters to measure. One might start by examining the abundance of facultative anaerobes relative to total bacteria using culture independent methods such as fluorescent in situ hybridization, and correlating this
abundance to nutrient levels and/or abundance of marine snow aggregates over time in a given environment. The results of this preliminary study are novel. Combined with more detailed studies suggested here, they may provide additional insight into the structure and function of heterotrophic bacterioplankton communities.

FIGURES

Figure 1: DAPI counts for sample stations in Bermuda-Woods Hole transect; note no error bars on station O3 due to problems with filter counting
Colony gallery

Cellobiose utilizing colony from the Sargasso: *Psychrobacter marincola*?

Glucose utilizing colony from Woods Hole Harbor

Casein utilizing colonies from the Sargasso

Chitin utilizing (?) colony from the Sargasso: *Brachybacterium paraconglomeratum*
Figure 2: Colony forming curves for BATS site, aerobic and anaerobic incubations

Figure 3: Colony forming curves from station O2, Sargasso NE of Bermuda
Figure 4: Colony forming curves from station O3, Sargasso anticyclone

Figure 5: Colony forming curves from station O6, Shelf Water
Figure 6: Colony forming curves from Woods Hole Harbor SW

Figure 7: Early/late colony fraction compared for aerobic and anaerobic incubations with same substrate and inoculum.
Figure 8: Location of each sampling station relative to SST measured by satellite on June 23, 2004. SST map obtained from [http://marine.rutgers.edu/mrg](http://marine.rutgers.edu/mrg).
Figure 9: Relationship of clones obtained in this study to cultivated isolates. Tree was constructed by adding short clone sequences with neighbor-joining to a previously calculated tree in ARB.
REFERENCES


