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Distribution of Capsulated Bacterioplankton in the North Atlantic and North Sea

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ABSTRACT

In laboratory experiments, bacterioplankton were incubated under different nutrient conditions, and the percentage of bacteria exhibiting a polysaccharidic capsule (capsulated bacteria) and that of CTC (cyanotetrazolium chloride)-positive and therefore metabolically highly active bacteria were determined. In these seawater cultures amended with nutrients more than 95% of the CTC-positive cells exhibited a capsule. During two cruises, one to the North Atlantic and one to the North Sea, we investigated the distribution of capsulated bacteria throughout the water column. Capsulated bacteria were generally more abundant in eutrophic surface waters than in deeper layers or more oligotrophic regions. In the upper 100 m of the North Atlantic, about 6–14% of the total bacterioplankton community was capsulated, while in the layers below 100 m depth, 97% of the bacteria lacked a visible capsule. The percentage of capsulated bacteria correlated with bacterial abundance and production, and chlorophyll *a* concentration. Also, the bioavailability of DOC (dissolved organic carbon), estimated by the ratio between bacterial production and DOC concentration, significantly correlated with the percentage of capsulated bacteria. In the North Sea, the contribution of capsulated bacteria to the total number of bacteria decreased from the surface (3 m depth) to the near-bottom (25–35 m) layers from 20% to 14% capsulated bacteria. In the nearshore area of the North Sea, about 27% of the bacteria exhibited a capsule. Overall, a pronounced decrease in the contribution of capsulated bacteria to the total bacterial abundance was detectable from the eutrophic coastal environment to the open North Atlantic. Using this epifluorescence-based technique to enumerate capsulated bacterioplankton thus allowed us to routinely assess the number of capsulated bacteria even in the oceanic water column. Based on the data obtained in this study we conclude that almost all metabolically highly active bacteria exhibit a capsule, but also some of the metabolically less active cells express a polysaccharide capsule detectable with this method.

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Introduction

Bacteria play an important role in the oceanic carbon cycle and exceed, in terms of biomass, phytoplankton in oligotrophic oceans even in the euphotic layer [2, 10]. Bacterioplankton are the only consumers of significance of marine dissolved organic carbon (DOC). DOC is taken up and a fraction of it is transformed into bacterial biomass while the major part is released as CO_2 into the ambient water. Because of the lack of reliable bacterial respiration measurements, especially under open ocean conditions, the actual growth yield of open water bacterioplankton is still under debate. Del Giorgio and Cole [5] estimated that bacterial growth yields for open water bacterioplankton could be <1%. About 10% of the DOC taken up by bacteria is released again as semilabile or refractory DOC into the ambient water via the formation and subsequent release of polysaccharidic capsular material [36].

Estimates of bacterioplankton biomass are most commonly obtained by enumerating and sizing of bacteria by epifluorescence microscopy [16]. This method, however, does not allow discrimination among metabolically active, dormant, or dead bacterial cells. There is some consensus now that not all of the bacteria present in the aquatic environment are metabolically active or alive [4, 23]. The reduction of tetrazolium salts to formazan (CTC method) by bacterial dehydrogenases is widely used to detect active, respiring bacteria [8, 35, 39]. Since a certain amount of formazan has to be built up before it can be detected, it has been concluded that only highly active bacteria are detectable with this method [8, 32]. Microautoradiography revealed that only about 2–50% of the 4′-6-diamidino-2-phenylindole (DAPI)-stained bacteria incorporate radiolabeled organic substrates [20, 26]. To visualize damaged, inactive, or dead cells a number of fluorochromes are now available, such as propidium iodide, ethidium bromide, or the BAG light kit [30]. Suzuki et al. [38] showed that bacteria without nucleoid-stainable structures [44] are capable of growth [see also ref. 1]. By comparing the above-mentioned methods, Karner and Fuhrman [20] found differences in the number of bacteria classified as active depending on the method used. Despite all these uncertainties, there is some general consensus emerging now that, on average, less than 30% of the bacterioplankton present are metabolically active [18, 20, 38, 39, 44].

Using transmission electron microscopy, Heissenberger et al. [14] found a highly significant relationship between intracellular integrity and the presence of a bacterial capsule. They showed that about 34% of the bacterial community had intact internal structures and as much as about 30% of the total bacterioplankton present in the euphotic zone of the Adriatic Sea exhibited a capsule.

In this study, we used laboratory experiments to test the hypothesis that capsulated bacteria are metabolically highly active. As an indicator of metabolically highly active bacteria we used the fluorescent tetrazolium salt 5-cyano-2,3-ditolyltetrazolium chloride (CTC) [32]. Furthermore, the distribution of capsulated bacteria was determined in the water column of the North Sea and the North Atlantic and related to parameters potentially influencing overall bacterial activity. For the enumeration of capsulated bacterioplankton, a recently developed light microscopy technique was used [37].

Materials and Methods

Laboratory Experiments to Concomitantly Determine Metabolically Active and Capsulated Bacteria

In order to test the hypothesis that capsulated bacteria are also metabolically active, we combined the capsule staining technique [37] to visualize bacterial capsules and the CTC (5-cyano-2,3-ditolyltetrazolium chloride) method to stain bacteria with high respiratory activity [7, 32]. Surface water of the coastal North Sea was sampled from the NIOZ pier. This freshly collected seawater was filtered twice through 0.8 μm or 0.2 μm polycarbonate (Millipore) filters to prepare two different sets of experiments: First, two seawater dilution cultures were prepared (0.2 μm:0.8 μm filtered seawater; ratio 9:1) and to one of the dilution cultures 70 mg L^{-1} colchicin and 150 mg L^{-1} cyclohexamide was added to prevent growth of eukaryotes. In the second set of experiments, 3 different cultures were established: one dilution culture (0.2 μm:0.8 μm filtered seawater; ratio 9:1), and two 0.8 μm filtered cultures, of which one culture was enriched with 50 μM glucose-C, 10 μM NH_4^+, and 1μM PO_4^{3-}; the other 0.8 μm filtered culture was left unamended. These different treatments were established in 2-L or 1-L flasks incubated in the dark at 20°C for 7 d and sampled at irregular intervals to determine the fraction of active and capsulated bacteria of the total bacterioplankton community using epifluorescence microscopy and flow cytometry (described in detail below).

Sampling for Assessing the Abundance of Capsulated Bacteria in the North Atlantic and North Sea

Water samples were collected during cruises at 2 different sites: the Faroe-Shetland Channel in the North Atlantic in July 1999 (1°W 62°N–6°W 60°N, R/V *Pelagia*) (Fig. 1a) and in the North

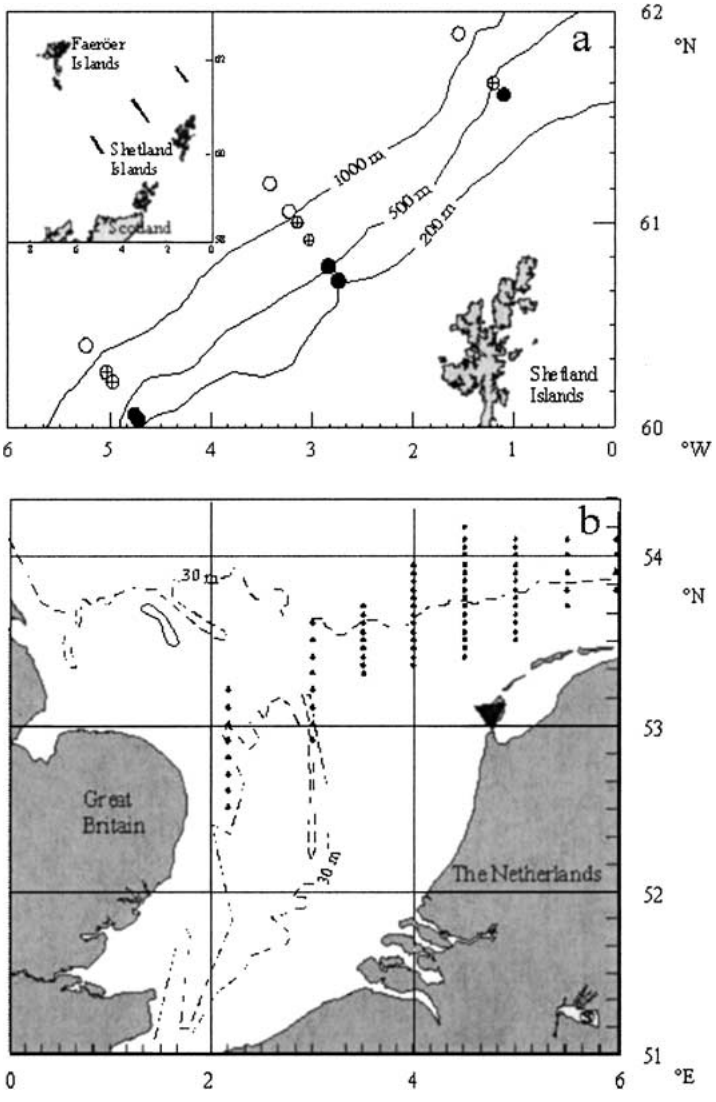

Fig. 1. Map of the study area in (a) the Faroe-Shetland Channel of the North Atlantic and (b) the southern North Sea with the sampling sites indicated by dots. In (a) ocean stations are marked by open circles, frontal stations by crossed circles, and shelf stations by full circles. The inset in (a) shows the location of the 3 transects sampled indicated by lines. The full triangle in (b) indicates the near-shore sampling site at the NIOZ pier on the island of Texel (The Netherlands), while the sampling sites in the open North Sea are indicated by full circles.

Sea in August/September 1999 (2°E 52°N–6°E 54°N, R/V *Mitra*) (Fig. 1b). Additionally, water from the coastal North Sea was collected at irregular intervals from the NIOZ pier during incoming tide (Marsdiep, Fig. 1b).

The Faroe-Shetland Channel was crossed from south to north in 3 transects (Fig. 1a) and is hydrographically characterized by 3 main water masses [33]. At the surface, relatively warm Gulf Stream water is flowing northwards. Between 500 and 800 m depth, low-salinity Arctic water flows southwards. Underneath this Arctic water, deep water originating from the Norwegian Sea is also flowing southwards. The Gulf Stream surface water is separated from the shelf waters by a well-defined front [33]. Water samples were taken during CTD hydrocasts at selected stations and depths with 10-L NEOX bottles mounted on a rosette.

In the North Sea, water samples were taken at selected stations from 3 m depth, midwater (15–25 m), and 3 m above the bottom (25–4495 m depth) (Fig. 1b).

Water samples (20 mL and 5 mL from the North Atlantic and the North Sea, respectively) were immediately fixed with 2% glutaraldehyde (final conc.) for later enumeration of the number of capsulated and noncapsulated bacteria. Furthermore, water was collected for bacterial enumeration after acridine orange or DAPI staining and for bacterial production measurements as described below.

Discrimination between Capsulated and Noncapsulated Bacteria

Generally, the protocol of Stoderegger and Herndl [37] was used to discriminate capsulated from noncapsulated bacterioplankton. Briefly, gelatin-coated slides were prepared by submerging them overnight in an ethanol (70%)–HCl (0.1 N) solution. Subsequently, the slides were coated by dipping them into a warm (60°C) solution of gelatin ($0.1\%^{v}/_{v}$) and $CrK(SO_4)_2{:}12H_2O$ ($0.01\%^{v}/_{v}$). After drying, the slides were stored frozen until used.

The water samples fixed with 2% glutaraldehyde were immediately filtered onto 0.2 μm polycarbonate filters (Poretics, 25 mm diameter) and rinsed with 0.5 mL of distilled water. Then, the filter was placed upside-down onto a 10 μL drop of 0.2 μm

filtered distilled water on the dry gelatin-coated slide and instantly frozen (−20°C) in a horizontal position. Slides were analyzed within 2 months. After thawing, the filter was allowed to air-dry completely. Then, the filter was peeled off from the glass slide and the area originally covered by the filter overlaid by a thin layer of 0.2 μm filtered 0.25% Congo Red solution (3–5 drops). Thereafter, the slide was allowed to dry at room temperature before it was overlaid by 3–4 drops of Maneval's stain (Carolina Biological Supply Company, NC, USA) for about 1 min. After rinsing with distilled water, the slide was gently blotted dry, fixed with paraffin oil, and examined under a phase contrast microscope (Zeiss, Axioplan) at 1250× magnification. The number of capsulated and noncapsulated bacteria was determined. All enumeration was done in duplicate by counting at least 300 bacteria per area originally covered by the filter.

The transfer efficiency of bacteria from the filter onto the slide was determined by filtering 5 mL of seawater onto a black polycarbonate filter (Millipore, 0.2 μm pore size, 25 mm filter diameter) after staining the bacteria with acridine orange. Subsequently, the bacteria were transferred onto a gelatin-coated slide (as described above) and the number of bacteria on the slide counted and compared with the number of bacteria adhering to the filter. The transfer efficiency of bacteria from the filter to the slide was 92.8% ± 4.3% (n = 20 slides).

Concomitant Determination of CTC-Positive Bacteria and Capsulated Bacteria

From each of the 5 different treatments established in the laboratory, 1 mL subsamples were withdrawn per sampling date and incubated in the dark at *in situ* temperature with a freshly prepared 100 μL CTC solution (5 mM final concentration) [32]. After 4 h, 100 μL of a 1% paraformaldehyde and 0.05% glutaraldehyde solution was added to stop the reduction of tetrazolium chloride to formazan. Thereafter, the sample was processed further following the capsule staining protocol as described above. Under the microscope, CTC-positive bacteria and capsulated bacteria were viewed concomitantly by switching between epifluorescence microscopy (for CTC-positive cells) and phase contrast microscopy (for capsulated cells) at 1250× magnification (Zeiss, Axioplan). As CTC-positive bacteria faded rapidly under the epifluorescence microscope, we also used flow cytometry for enumeration of CTC-positive bacteria. For flow cytometric analysis, 50 μL CTC solution (5 mM final concentration) was added to 0.5 mL subsamples and after 4 h the incubation was terminated by adding 50 μL of 1% paraformaldehyde and 0.05% glutaraldehyde.

Flow Cytometry

The Epics Elite flow cytometer (Coulter ESP), equipped with an argon laser (wavelength: 488 nm; power level: 15 mW), was calibrated with 10 μm diameter fluorescent beads. A 525 nm bandpass filter was used for the specific detection of PicoGreen-stained cells, while a 675 nm long-pass filter was used for CTC-formazan fluorescence. A flow rate of ∼50 μL min^{-1} (running time: 4 min) was applied. Data were recorded in list mode and later analyzed on a separate workstation using the Elite software program.

For total bacterial abundance, 1 mL samples were preserved with 100 μL 1% paraformaldehyde and 0.05% glutaraldehyde and after 10 min frozen in liquid nitrogen and stored at −40°C. Samples for enumeration of CTC-positive cells were frozen in liquid nitrogen and processed within 2 h; however, storage of samples for up to 3 weeks (−40°C) is possible without a significant change in the number of CTC-positive cells (data not shown).

Bacterial abundance was determined on samples with a final concentration of PicoGreen of 1:100 of the stock solution [42]. A blank (0.2 μm filtrate) was prepared separately for each sample and subtracted from the sample. The blank was generally <10% of the bacterial abundance of the sample.

Bacterial Production

During the cruise in the North Atlantic, bacterial production was measured via the incorporation of [^{3}H]thymidine (specific activity: 81 Ci $mmol^{-1}$, Amersham; final concentration 20 nM) into bacterial DNA [9] and [^{14}C]leucine (specific activity: 0.315 Ci $mmol^{-1}$, Amersham; final concentration, 10 nM) into bacterial protein [34] using the dual labeling approach [18]. Triplicate subsamples of 10–20 mL and 1 formaldehyde-fixed control (2% formaldehyde final conc.) were incubated in the dark for 60–120 min. After incubation, the subsamples were fixed with formaldehyde and filtered onto 0.2 μm cellulose nitrate filters (Millipore, 25 mm diameter). The filters were rinsed 3 times with 10 mL of ice-cold 5% trichloroacetic acid (Sigma Chemicals). The filter was dissolved in 1 mL ethyl acetate (Riedel de Haen) and after 10 min, 8 mL of scintillation cocktail (Insta-Gold, Canberra Packard) was added. After 18 h, the radioactivity collected on the filter was determined using a liquid scintillation counter (Wallac LKB 1211, Rackbeta). The disintegrations per minute (DPM) were converted into the actual amount of substrate incorporated into macromolecules using the conversion factors of 2×10^{18} cells produced mol^{-1} thymidine [10] and 0.08×10^{18} cells mol^{-1} leucine [34].

Enumeration of the Total Bacterial Abundance

For all samples where CTC-positive cells were determined, total bacterial abundance was assessed by flow cytometry using the double-stranded DNA stain PicoGreen (Molecular Probes, Inc.).

To determine the total bacterial abundance in samples where no CTC activity was assessed and for the enumeration of capsulated and noncapsulated bacteria, light microscopy was applied. Total bacterial abundance was determined after staining 5 mL or 20 mL of water with acridine orange [17] or DAPI (4′-6-diamidino-2-phenylindole; [31]) and filtering it through black polycarbonate filters (Millipore, 0.2 μm pore size, 25 mm diameter). At least 300 bacteria per filter were counted and sized under the epifluorescence microscope (Zeiss, Axioplan) at 1250× magnification. The bacterial volume was converted into bacterial C-biomass by using the allometric formula given in Lee and Fuhrman [21].

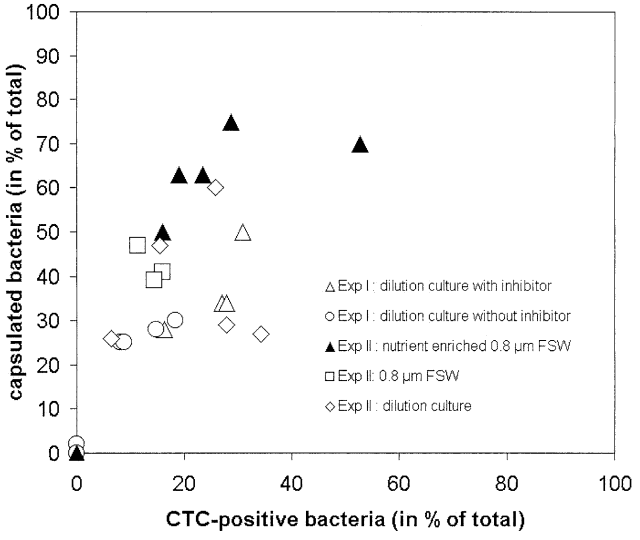

Fig. 2. Relation between CTC-positive and the percentage of capsulated bacteria obtained in the 5 different culture treatments (details on culture treatments are given in Materials and Methods).

Results

Relation between CTC-Positive Bacteria and the Presence of a Capsular Envelope

To test whether CTC-positive bacteria were capsulated, seawater cultures with natural bacterioplankton communities were established under different nutrient conditions. Subsamples of these cultures were double-stained with CTC and the negative staining technique for capsules [37]. Using this combination, >95% of the CTC-positive bacteria exhibited a capsule. However, because of the rapid fading of CTC-positive cells when illuminated, enumeration and classification of cells was not done on a routine basis. Enumeration first by transmission microscopy to determine the number of capsulated bacteria and subsequently, on another subsample, by flow cytometry to enumerate the CTC-positive cells allowed us, however, to determine both categories of cells. The percentage of capsulated bacteria obtained for the different experiments and treatments correlated with that of CTC-positive bacteria (regression analysis, $p < 0.001$, $r^2 = 0.6$; $n = 27$, Fig. 2).

In the dilution cultures, the percentage of capsulated and CTC-positive bacteria was highest in the logarithmic growth phase (shortly before highest bacterial abundance was reached) and decreased after total bacterial abundance reached its maximum in the different treatments. In the culture where flagellate grazing was inhibited, the percentage of CTC-positive bacteria was, on average, twice as high as in the culture where no eukaryotic inhibitors were added (Wilcoxon; $p = 0.04$; $n = 6$). In the nutrient-amended culture, the percentage of capsulated and of CTC-positive bacteria reached highest values with 52% CTC-positive and 75% capsulated bacteria after 48 and 72 h, respectively (Fig. 2).

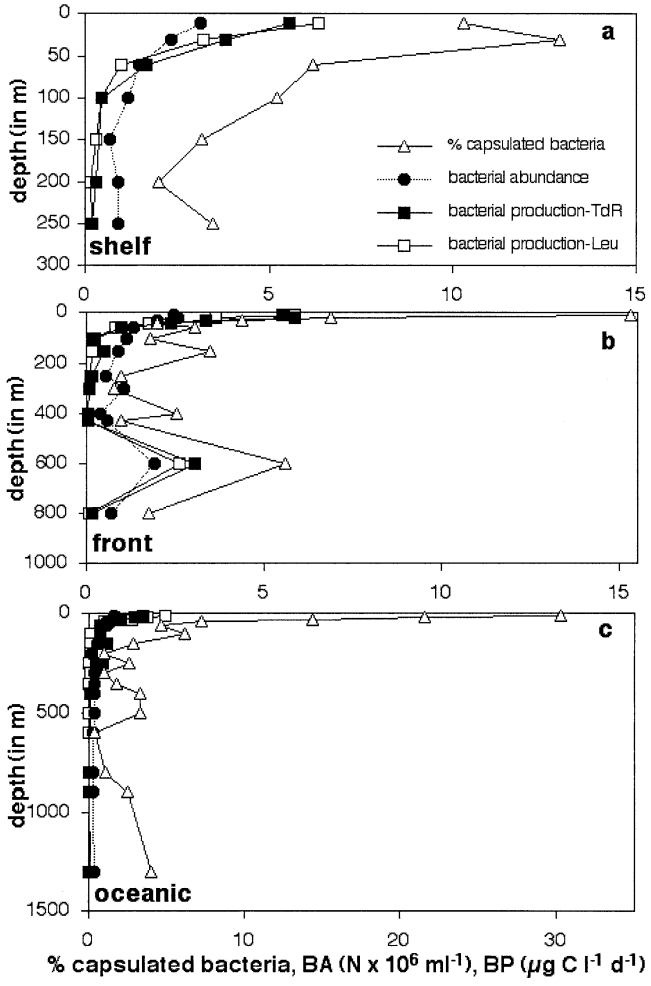

Fig. 3. Depth profiles of the percentage capsulated bacteria, bacterial abundance (BA), and bacterial production (BP; in µg C $L^{-1}d^{-1}$) determined by thymidine (TdR) and leucine (Leu) incorporation of the Faroe-Shetland Channel in (a) shelf waters, (b) the frontal system, and (c) the open waters. Symbols represent the mean of at least 4 stations sampled at the specific depths (each sampling site was sampled 1–5 times).

Distribution of Capsulated Bacteria in the Water Column of the North Atlantic

In the Faroe-Shetland Channel of the North Atlantic (Fig. 1a), the percentage of capsulated bacteria decreased from 5 m depth to 100 m depth (Fig. 3). Below 100 m depth, the percentage of capsulated bacteria was rather constant with

Table 1. Pearson's correlation coefficients (r) between the percentage of capsulated bacteria and selected parameters in the North Atlantic at the shelf (n = 23–25), frontal (n = 22), and oceanic stations (n = 30), all North Atlantic stations pooled (n = 75–77), and for the North Sea (all station pooled; n = 186).

Variable	Shelf	Front	Oceanic	North Atlantic (pooled)	North Sea (pooled)
Bacterial abundance mL^{-1}	0.48	NS	0.73	NS	0.14*
BB (μg C L^{-1})	0.41	0.63	0.66	0.40	n.d.
$BP_{thymidine}$ (μg C L^{-1} d^{-1})	0.50	0.67	0.74	0.49	n.d.
$BP_{leucine}$ (μg C L^{-1} d^{-1})	0.38*	0.80	0.66	0.52	n.d.
Chlorophyll *a* (μg L^{-1})	0.47	0.60	0.65	0.55	0.15
DOC (μM)	0.38*	NS	NS	NS	NS
PO_4 (μM)	−0.40*	−0.65	−0.74	−0.52	−0.16
NO_3 (μM)	−0.41	−0.60	−0.76	−0.53	−0.31

[a] Only correlations with $p < 0.05$ are shown, $p < 0.1$ indicated by *. NS, not significant; n.d., not determined; BB, bacterial biomass; BP, bacterial production.

2.2 ± 1.1% (shelf, front, and oceanic stations pooled). The percentage of capsulated bacteria decreased between the upper 100 m layer and the deeper layer (>100 m) at both the frontal and shelf stations approx. 3-fold, while at the oceanic stations this decrease was more pronounced (about 6.5 fold; Fig. 3).

Bacterial production in surface waters (0–100 m depth), based on thymidine incorporation, ranged from 2.1 ± 1.1 μg C L^{-1} d^{-1} at the oceanic stations to 3.0 ± 2.3 μg C L^{-1} d^{-1} at the frontal stations. Based on leucine incorporation, bacterial production of the 0–100 m water column ranged from 2.3 ± 1.9 μg C L^{-1} d^{-1} to 2.7 ± 2.7μg C L^{-1} d^{-1} from the ocean to the shelf site, respectively. For thymidine incorporation, a 13-fold, 18-fold, and 5-fold decrease from surface (0–100 m depth) to deep waters (>100 m) was detectable for shelf, frontal, and oceanic waters, respectively. Leucine incorporation into bacteria decreased in a more pronounced manner from the top 100 m layer to the water layers below 100 m depth (14-, 32-, and 46-fold for shelf, frontal, and oceanic water, respectively).

If data from all the stations were pooled, the percentage of capsulated bacteria only weakly correlated with bacterial production for both thymidine and leucine incorporation (Pearson; $p < 0.01$; n = 61–77; Table 1, Fig. 4). No correlation was detectable between the percentage of capsulated bacteria and bacterial abundance if data from all the stations were pooled. At the shelf stations, the correlation between the percentage of capsulated bacteria and bacterial production was weakest (Pearson; $p = 0.06$ for leucine; $p = 0.01$ for thymidine; $n = 25$) while total bacterial abundance correlated with the percentage of capsulated bacteria (Pearson; $p = 0.015$; $n = 25$)(Table 1). At the oceanic stations, bacterial abundance and thymidine- and leucine-based bacterial production correlated with the percentage of capsulated bacteria (Pearson; $p < 0.001$; $n = 30$) (Table 1) more closely than for the shelf and frontal site.

For all three areas pooled, the percentage of capsulated bacteria was positively correlated with bacterial biomass (Pearson; $p < 0.05$ at least)(Table 1).

Vertical and Horizontal Distribution of Capsulated Bacteria in the Southern North Sea

During August/September 1999, the percentage of capsulated bacteria was, on average, 19.6 ± 10.4% (n = 62) for the surface layers of the southern North Sea and significantly lower in the near-bottom water samples (3 m above bottom, 14.4 ± 7.4%, Bonferroni; $p < 0.05$, n = 62)(Fig. 5). The percentage of capsulated bacteria in the midwater samples was, on average, 17.6 ± 9.1% and therefore not significantly different from both the surface and bottom water samples (Bonferroni; $p > 0.05$ level; $n = 60$). Mean bacterial abundance varied only within a small range throughout the water column (1.3 ± 0.4 × 10^6 cells mL^{-1}) with no significant difference in abundance detectable between the three layers (Fig. 5). The percentage of capsulated bacteria was weakly correlated with bacterial abundance at both sampling sites of the North Sea, the open southern North Sea (Pearson; $p = 0.052$; $n = 186$), and the coastal site (Pearson; $p = 0.052$; $n = 25$) (Table 1).

General Trends in the Distribution of Capsulated Bacteria

Generally, the percentage of capsulated bacteria was much higher in surface than in deeper waters. Integrated over

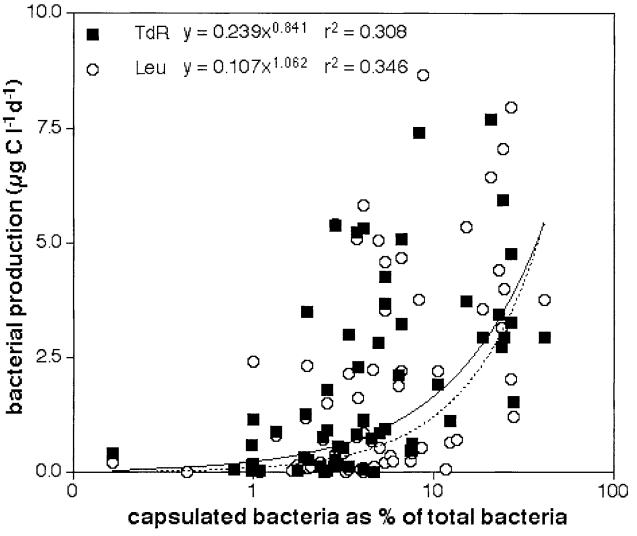

Fig. 4. Relation between the percentage capsulated bacteria and bacterial production estimated via thymidine (filled squares, solid line) and leucine (open circles, broken line) incorporation (data from the entire sample area are shown).

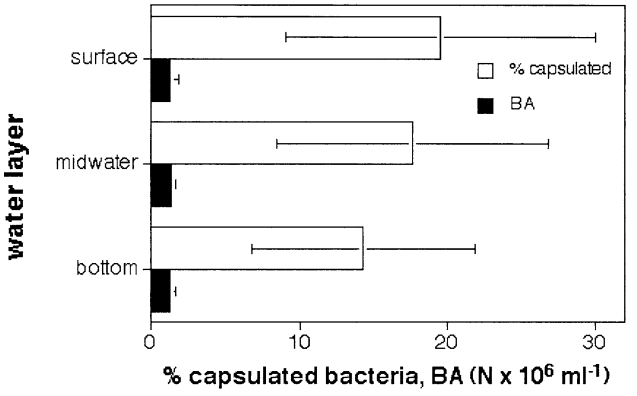

Fig. 5. Distribution of the percentage of capsulated bacteria (open bars) and bacterial abundance (BA, dark bars) in the three depth layers sampled in the southern North Sea. Bars represent mean ± SD (n = 62) of BA of three depth layers sampled. Surface: 3 m depth; midwater: 15–25 m; near bottom water: 25–45 m depth. The percentage of capsulated bacteria in the surface layer is significantly higher than in the near-bottom waters (Bonferroni; $p < 0.05$; n = 62); bacterial abundance is not significantly different among the three depth layers sampled (Bonferroni; $p > 0.05$; n = 60).

the water column, the distribution of the percentage of capsulated bacteria significantly increased (Bonferroni; $p < 0.05$) from the open North Atlantic (7% capsulated bacteria) to the southern North Sea (~17% capsulated bacteria) (Table 2). In the nutrient-rich near-shore waters of the North Sea, on average, 27% of capsulated bacteria were detectable, indicating an overall increase of capsulated bacteria from open to near-shore, nutrient-rich waters (Table 2). Comparing different depth layers of the open North Atlantic, 9.4 ± 9.1% capsulated bacteria were present in the 0–100 m layer, decreasing to 2.9 ± 1.7% in the 100–250 m depth layer; below 250 m, 2.0 ± 1.2% of the bacteria were capsulated (Table 2).

Discussion

In the present study we applied a recently developed light-microscopy based method [37] to enumerate capsulated bacteria. We combined this method with the assay to enumerate CTC-positive bacteria to simultaneously determine the number of metabolically highly active (by the criteria of Sherr et al. [32]) and capsulated bacteria. The percentage of CTC-positive cells in natural waters is usually low, ranging from 0.1% for deeply mixed waters and 1–2% for surface mixed waters in the Gulf of St. Lawrence [24] to about 7% in coastal areas [6, 11]. However, using microautoradiography, Tabor and Neihof [39] found 4–61% of metabolically active bacteria in marine system. In a detailed study on the potential of CTC as an indicator for bacterioplankton activity, Sherr et al. [32] and Choi et al. [3] concluded that only metabolically highly active bacteria develop sufficient fluorescence when stained with CTC–formazan to allow enumeration while less active cells remain unstained. These authors also showed that with the addition of substrate and an increase in temperature, the number of CTC-positive cells increases by at least one order of magnitude [3, 32]. This corresponds well with our findings where, upon nutrient addition, the percentage of CTC-positive cells increased to up to 50% of the total bacteria enumerated with DAPI or acridine orange.

Relation between Capsulated and CTC-Positive Bacteria

More than 95% of the metabolically highly active (CTC-positive) bacteria also exhibited a capsule as determined by examination on a single cell level (by switching between transmission and epifluorescence microscopy). The rapid fading of CTC-positive cells under the microscope could be circumvented by using flow cytometry, where a significant correlation between the percentage of capsulated and CTC-positive bacteria was obtained (Fig. 2). The number of capsulated bacteria was higher than the number of CTC-positive bacteria (Fig. 2) implying that not all

Table 2. Capsulated bacteria as percentage of total bacteria and total bacterial abundance in different water masses averaged over sampling days and depth layers.

Sample station (period)	Water type	% capsulated bacteria	Bacterial abundance (10^{6+}mL^{-1})	*n*
North Atlantic (July 99)	Oceanic-shelf	7.0 ± 8.1	1.3 ± 0.8	80
North Atlantic (0–100m)	Surface	9.4 ± 9.1	1.7 ± 0.7	51
North Atlantic (100–250m)	Deep	2.9 ± 1.7	0.7 ± 0.3	17
North Atlantic (>250m)	Deep	2.0 ± 1.2	0.5 ± 0.2	12
North Sea (September 1999)	Shelf	17.2 ± 9.2	1.3 ± 0.4	186
Near-shore North Sea (June 1999)	Near-shore	26.7 ± 5.5	9.1 ± 1.5	23

[a] The period of investigation or the water layer is given in brackets. Numbers represent the mean ± SD; *n* is the number of samples enumerated. The percentages of capsulated bacteria of the North Atlantic, the North Sea, and the near-shore waters of the North Sea were all significantly different from each other (Bonferroni; $p < 0.001$, for all combinations).

the capsulated bacteria were CTC-positive, i.e., metabolically highly active.

Distribution of Capsulated Bacteria in the Water Column of the North Atlantic and the North Sea

Integrated over the water column, the percentage of capsulated bacteria increased from more oligo- to more eutrophic conditions and therefore, with overall metabolic activity. The distribution of capsulated bacteria significantly increased from the open North Atlantic (Table 2) and from deeper to surface waters (Fig. 3).

As an estimate of the bioavailability of DOM, the ratio between bacterial production and DOC concentration was calculated [29] and related to the percentage of capsulated bacteria in the open North Atlantic. A significant correlation (Pearson; $p < 0.01$, $n = 22$–30) was detectable for the pooled data set and the different sites, except for the shelf, where only a weak correlation was detectable (Pearson; $p < 0.1$, $n = 25$, for thymidine as well as for leucine). This suggests that if DOM is more bioavailable (as indicated by a high bacterial production:DOC ratio) the percentage of capsulated bacteria increases.

Why Do Highly Active Bacteria Express a Capsule?

In this study we were able to show that >95% of all the metabolically active bacteria exhibit a capsule and that there is a clear relation between the numbers of CTC-positive and capsulated bacteria (Fig. 2). As different culture conditions all led to similar trends (Fig. 2), we assume that the obtained relation is not the result of selecting for a specific bacterial species since different nutrient regimes would probably favor growth of different species. Moreover, a higher percentage of capsulated bacteria was found under *in situ* conditions when bacterial metabolism was also higher (Table 1, Figs. 3, 4).

Is the capsule a by-product or waste product of bacterial metabolism or is it produced to enhance nutrient assimilation, prevent viral attack, or increase the ability to attach to particles? Gurijala and Alexander [13] found enhanced grazing on bacterial species with a less hydrophobic cell surface. Monger et al. [27], however, showed that picoplankton with increased hydrophobicity are more efficiently grazed by flagellates and that the hydrophobicity of the cells increased from oligotrophic to mesotrophic conditions and decreased from the surface to deeper layers. In contrast, bacterial surface charge did not influence prey selection of three common species of nanoflagellates feeding on 14 different bacterial strains [19].

Up to now, little research has been done on the role of the capsule in marine bacterioplankton. From medical research it is known that the bacterial capsule consists of polysaccharides either with one hydrophobic region at one end of the polysaccharide molecule (the type known as amphiphilic polymers) or with hydrophobic groups distributed across the entire polysaccharide (polyphilic polymers). Probably the best known examples of bacterial polyphilic polysaccharides are the 6-deoxy sugars rhamnose and fucose, as well as *N*-acetylhexosamines [28].

Bacteria were found to become more hydrophobic during the exponential growth phase or at high growth rates in chemostats [25, 40]. With increasing hydrophobicity, an increased tendency to adhere to particles or to the air–water interface was found [15], accompanied by a stimulated synthesis of exopolymers [41]. Production of exopolymers, and hence a capsule, might also be a response to nutrient depletion and a result of the starvation survival program [43]. However, it has also been shown that streptococci are

able to produce an uncharged extracellular polysaccharide matrix in response to nutrient addition [12]. Thus, findings on the environmental conditions regulating hydrophobicity of bacterial cell surfaces are contradicting at present. Lemke et al. [22] showed that hydrophobic bacteria enter the exponential growth phase earlier than hydrophilic bacteria. It seems likely that bacteria are more hydrophobic under nutrient-replete conditions [40] either because of generally increased hydrophobicity of the entire bacterial community or because of an increased dominance of specific, more hydrophobic species.

Summarizing, we may conclude that the formation of a capsule seems to be an important mechanism for a metabolically active bacterium to interact with its environment. In this study we showed, that the expression of a capsule is a function of the metabolic activity of the bacterium, being enhanced under nutrient-replete conditions. A direct correlation of the percentage of capsulated bacteria to overall bacterial growth and phytoplankton biomass was detectable. One might speculate that the expression of a capsule facilitates nutrient scavenging of bacteria by increasing the surface area of the cells when sufficient nutrients are available. Clearly, further research is required to fully elucidate the ecological role of the capsule in bacterioplankton.

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