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Growth Patterns of Two Marine Isolates: Adaptations to Substrate Patchiness?

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During bottle incubations of heterotrophic marine picoplankton, some bacterial groups are conspicuously favored. In an earlier investigation bacteria of the genus *Pseudoalteromonas* rapidly multiplied in substrate-amended North Sea water, whereas the densities of *Oceanospirillum* changed little (H. Eilers, J. Pernthaler, and R. Amann, Appl. Environ. Microbiol. 66:4634–4640, 2000). We therefore studied the growth patterns of two isolates affiliating with *Pseudoalteromonas* and *Oceanospirillum* in batch culture. Upon substrate resupply, *Oceanospirillum* lagged threefold longer than *Pseudoalteromonas* but reached more than fivefold-higher final cell density and biomass. A second, mobile morphotype was present in the starved *Oceanospirillum* populations with distinctly greater cell size, DNA and protein content, and 16S rRNA concentration. Contrasting cellular ribosome concentrations during stationary phase suggested basic differences in the growth responses of the two strains to a patchy environment. Therefore, we exposed the strains to different modes of substrate addition. During cocultivation on a single batch of substrates, the final cell densities of *Oceanospirillum* were reduced three times as much as those *Pseudoalteromonas*, compared to growth yields in pure cultures. In contrast, the gradual addition of substrates to stationary-phase cocultures was clearly disadvantageous for the *Pseudoalteromonas* population. Different growth responses to substrate gradients could thus be another facet affecting the competition between marine bacteria and may help to explain community shifts observed during enrichments.

Prefiltration and confinement of marine bacterioplankton during enrichments (8, 43), dilutions (13), and enclosure experiments (36, 37) can result in changes of both taxonomic composition and phenotypic features of communities. The percentage of cells with higher per-cell rRNA, DNA, and protein content (8, 13, 15), the proportion of plate-countable cells (11), and the proportion of cells exhibiting higher metabolic activity (15, 41) have all been observed to increase. Often the original community is overgrown by a few genera of frequently cultured marine gamma-proteobacteria, e.g., *Vibrio* sp., *Alteromonas* sp., and *Pseudoalteromonas* sp. (8, 16, 37), which are, however, most probably not very abundant members of the bacterioplankton (8).

Are those microbes that are not enriched in bottles or enclosures in principle unable to grow on the offered substrates? The majority of pelagic bacteria and archaea are capable of incorporating mixes of radiolabeled amino acids (21, 29). In previous works, strains related to the genera *Roseobacter* (alpha-proteobacteria), *Oceanospirillum* sp. (gamma-proteobacteria), and *Cytophaga* sp. (*Bacteroidetes*), were isolated from North Sea water samples on a substrate mix of amino acids and monomers, yet members of these lineages were not enriched from North Sea plankton during incubations of filtrates on the same substrates (8, 9).

Bacterioplankton community change upon filtration and/or substrate addition may thus be a consequence of other features of the enriched populations, rather than of the ability to utilize a particular substrate. A considerable proportion of the substrates and bacterial productivity in coastal pelagic environments are distributed in microscale patches of variable concentration and size, such as algal "phycospheres," marine snow, or metazoan fecal pellets (3, 31). The particle-attached and free-living pelagic communities differ both in phenotypes and in taxonomic composition (1, 7). Individual microbial species or phylogenetic lineages within the bacterioplankton may consequently differ in their ability to succeed in habitats with steeper or flatter substrate gradients. We therefore hypothesized that bacteria which exhibit a more rapid growth response under batch culture "feast-and-famine" conditions (32) are also favored during enrichments of environmental samples.

Flow cytometry and image-analyzed epifluorescence microscopy are tools to study growth-related microbial cell features, e.g., size and macromolecular content, both in whole communities (13, 14, 25, 33, 45) and in individual populations (5, 20). For example, a high per-cell ribosome content is generally regarded as a feature of active bacteria in mixed assemblages (2). Pure culture studies show a dependence of total ribosome content on growth rate in continuous cultures (5, 22, 26, 34). Furthermore, it has been suggested that some bacteria maintain a high rRNA content (i.e., excess protein synthesis capacity) during nongrowth to be able to rapidly respond to changes in growth condition (10, 12). If this hypothesis is correct, bacterial strains that exhibit contrasting patterns of per-cell ribosome concentration during early stationary phase should also differ in their competition for more or less patchy substrates.

Batch growth experiments with two marine isolates were performed in pure culture and coculture on low concentrations of organic carbon. The selected strains are affiliated with gamma-proteobacterial genera that had exhibited contrasting responses during substrate-amended enrichments of environmental samples in an earlier study (8) (Table 1). Cell numbers and sizes and the patterns of rRNA, total nucleic acid, and protein content per cell were followed during the different

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TABLE 1. Abundances of *Pseudoalteromonas* sp. and *Oceanospirillum* sp. in enrichments of North Sea filtrates and FISH detectability of the two studied strains during long-term starvation (for details, see reference 8)

Species used	Abundances in enrichments (10^5 cells ml^{-1}) (mean ±SD) during incubation period of:			FISH detectability during starvation (% of total cells) (mean ± range) during incubation period of:		
	0 h	24 h	48 h	0 days	35 days	53 days
Pseudoalteromonas sp.	b.l.[a]	b.l.	3.28 (±0.40)	100 (±1.7)		71 (±28.4)
Oceanospirillum sp.[b]	0.06 (±0.2)	0.33 (±0.72)	0.44 (±0.32)	93 (±1.4)	4 (±0.6)	4 (±2.6)

[a] b.l., below limit for FISH counting (<1% total counts).
[b] *Oceanospirillum* sp. data from reference 8.

growth phases in pure cultures. The population sizes of the two strains were then monitored in cocultures to which substrates were either added in one batch or gradually.

MATERIALS AND METHODS

Batch cultures of single strains. Growth experiments were carried out on a synthetic medium previously used for ecophysiological investigations on a marine *Sphingomonas* sp. (39). A mix of monomers and amino acids as described by Eilers et al. (9) was added to the medium at micromolar concentrations. The two marine strains used in this study, *Oceanospirillum* sp. strain KT0923 and *Pseudoalteromonas* sp. strain KT0912.10 (45), were both isolated from surface waters in the German Bight of the North Sea (9). According to 16S rDNA gene sequence analysis, they are phylogenetically most closely affiliated with *Oceanospirillum commune* and *Pseudoalteromonas atlantica* (95.7 and 99.7% rDNA similarity, respectively). Prior to the experiments, both strains were maintained on liquid medium for several growth cycles. Six days after their last reinoculation, 4 liters of freshly prepared medium was inoculated at initial densities of approximately 10^5 cells ml^{-1}. Incubations were performed in two parallels at 15°C and with gentle stirring. Fifty-ml subsamples were taken at 30-min to 2-h intervals for the first 58 h and at longer intervals thereafter, were fixed for 30 min with formaldehyde solution (final concentration, 2% [vol/vol]), and were stored frozen (−80°C) until further processing.

For the competition experiments, strains were inoculated at densities of approximately 0.5×10^5 cells ml^{-1}. In one set of treatments ("batch cocultures"), substrates were present in the medium at the time of inoculation. In a second set ("extended batch cocultures"), portions of the substrate mix (1% of total) were added hourly to the medium by a peristaltic pump. In addition, two controls without substrates were inoculated with the two strains. Subsamples were aseptically taken at several time points and were treated as described above.

Flow cytometry. Samples were analyzed on a FACStar Plus flow cytometer (Becton Dickinson, Mountain View, Calif.). Cell counts and DNA and protein quantifications were carried out as previously described by simultaneous staining with the fluorescent dyes HOECHST33342 and SYPRO (Molecular Probes, Leiden, The Netherlands) and by double excitation with UV and green lasers (265 and 543 nm) (27, 45). Fluorescence was measured with logarithmic signal amplification. All measurements were standardized to the fluorescence of latex beads (FluoroSpheres, yellow green, 2-μm diameter; Molecular Probes) added to each sample at known concentrations. Absolute bacterial abundances were determined from the ratios of beads to bacteria. Objects that showed both DNA and protein fluorescence above background levels were regarded as bacteria. At least 2,000 such positive events, excluding beads, were recorded per sample. To avoid errors due to clustering of cells, samples were sonicated for 5 s prior to measurements (OmniLab sonicator bath; Bandelin, Berlin, Germany). Depending on cell concentration, data were acquired for a few seconds to several minutes. Measurements were excluded from the evaluation of fluorescence intensities if a significant drift of signal during the acquisition period was detected. Analysis of samples from the first experimental vessel revealed instrument instabilities; therefore, DNA and protein fluorescence intensities were evaluated from samples of the second experimental vessel only. The relative number of events in the high- and low-DNA subpopulations was determined for time points when two separate maxima of DNA fluorescence were readily distinguishable in histogram plots. Within the DNA-rich cell fraction of *Oceanospirillum* sp. populations, the frequency of bacteria with a high or low protein content was quantified during lag phase.

FISH. Based on the flow cytometry counts, selected time points of the growth curves were analyzed by fluorescence in situ hybridization (FISH). Subsamples were filtered onto white membrane filters (GTTP, diameter, 47 cm; pore size, 0.2 μm; Millipore, Bedford, Mass.) and were hybridized with the CY3-labeled probe EUB338 (2) for quantitative FISH. Specific probes for *Pseudoalteromonas* sp. and *Oceanospirillum* sp. (9) were used to evaluate the competition experiment. Hybridization and washing buffers were composed as described previously (9, 17). To minimize differences between quantitative hybridizations, the handling time between incubation and washing was standardized. All filter sections from a complete time series were hybridized simultaneously in one single batch of hybridization buffer. Samples were air dried and embedded in VectaShield antifading mounting medium (Vector Laboratories, Burlingame, Calif.).

Image acquisition and analysis. Gray images of fluorescently labeled cells were acquired at ×100 magnification on a confocal laser scanning microscope (LSM 510; Carl Zeiss, Jena, Germany) (calibrated pixel length, 0.064 μm; 4,096 gray levels). Since the stability of a laser as excitation light source is superior to that of a mercury arc bulb, conditions of measurement setups are more readily reproduced. Probe fluorescence from excitation with a green laser (HeNe, 543 nm) was recorded at a scanning speed of 30 s. To ensure output stability, the laser was switched on at least 2 h prior to measurements. To minimize uncontrolled cell bleaching, microscopic focusing was carried out by rapid prescanning at low laser intensity rather than by illumination with the mercury arc bulb. Background fluorescence was excluded by appropriate adjustment of the pinhole, which was set to collect light from a 0.6-μm-thick optical section (corresponding to the average cell width). This optical sectioning, moreover, provided an efficient focusing aid during prescanning, as even small deviations from the optimal focal position resulted in a strong decrease of cell brightness. We avoided all microscopic fields in which brightness gradients of stained cells were apparent, because such gradients probably indicated that the respective filter positions were not sufficiently horizontal for brightness measurements within a 0.6-μm slice. A total of 300 to 1,000 individual cells from 10 to 20 images was analyzed per sample.

Images were processed and measured with the software MetaMorph (version 3.5; Universal Imaging, West Chester, Pa.). Object edges were established by Unsharp Masking (28). The gray image was smoothed by a 16- by 16-pixel square, low-pass kernel, downscaled to 95% of its original brightness, and subtracted from the original image. The resulting image was multiplied by 20, and noise was reduced by a 5- by 5-pixel neighborhood Median filter. The edge-enhanced images from a series were subsequently thresholded automatically at a preset intensity (gray value, 200 to 500). The binary image served as a mask for size and brightness detection. Edges were smoothed by morphological closing, and objects of <25 pixels and of >1,000 pixels were discarded. Each processed image was examined and if required was interactively edited prior to measurement (exclusion of irregularly shaped objects and separation of touching cells). Object area, perimeter, total, and mean gray values were recorded. Cell volumes were calculated from the measured area and perimeter (33). To compensate for potential differences between individual hybridization series, a sample from a time point with a low standard deviation of mean gray values (*Pseudoalteromonas* sp., parallel 2, 100 h) served as the internal standard. In each series of samples, this internal standard was also hybridized and evaluated and brightness values from different hybridization series were corrected accordingly.

RESULTS

Pure culture batch growth. Following transfer to fresh medium, the lag phase of *Pseudoalteromonas* sp. (defined as the period between inoculation and the first doubling of cell numbers) was significantly shorter (9 [±1] h) than that of *Oceanospirillum* sp. (25 [±1] h) (Fig. 1). The highest doubling times of *Pseudoalteromonas* sp. and *Oceanospirillum* sp. were 2.4 and 3.7 h, respectively. The *Pseudoalteromonas* sp. population ceased cell division after 27 (±1) h, *Oceanospirillum* sp. after

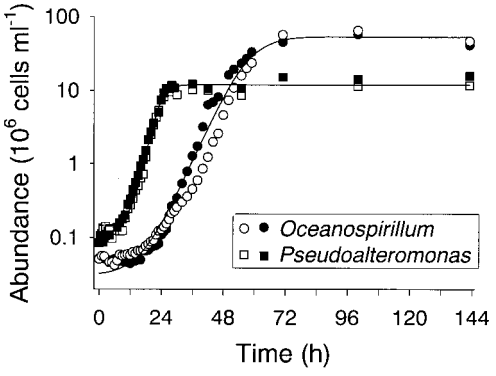

FIG. 1. Batch growth of *Oceanospirillum* sp. and *Pseudoalteromonas* sp. in two separate experiments.

100 h. At the onset of stationary phase, *Pseudoalteromonas* sp. had 18% of the cell density and 17% of the biomass of *Oceanospirillum* sp.

Relative 16S rRNA concentration per cell. Mean per-cell fluorescence after quantitative FISH with the 16S rRNA-targeted probe EUB338 was used to estimate changes in rRNA concentrations of *Oceanospirillum* sp. and *Pseudoalteromonas* sp. during growth (Fig. 2). Both organisms showed an increase of rRNA content before significant cell multiplication was detectable, and maximal RNA fluorescence intensity was approximately double its initial value in both strains. This maximum occurred during late logarithmic growth in *Oceanospirillum* sp. and at the onset of stationary phase in *Pseudoalteromonas* sp. The relative per-cell rRNA concentration of *Pseudoalteromonas* sp. was significantly elevated during 100 h of nongrowth. In

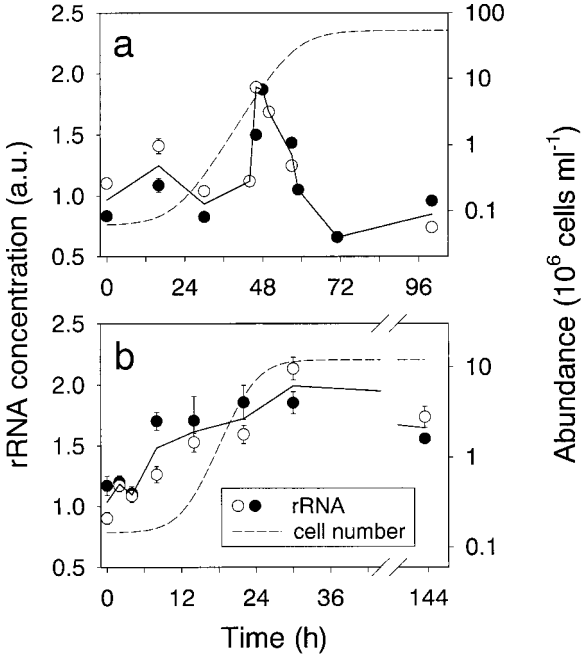

FIG. 2. Mean 16S rRNA concentration of *Oceanospirillum* sp. (a) and *Pseudoalteromonas* sp. (b) of two separate experiments (means ± 1 standard error). a.u., arbitrary units.

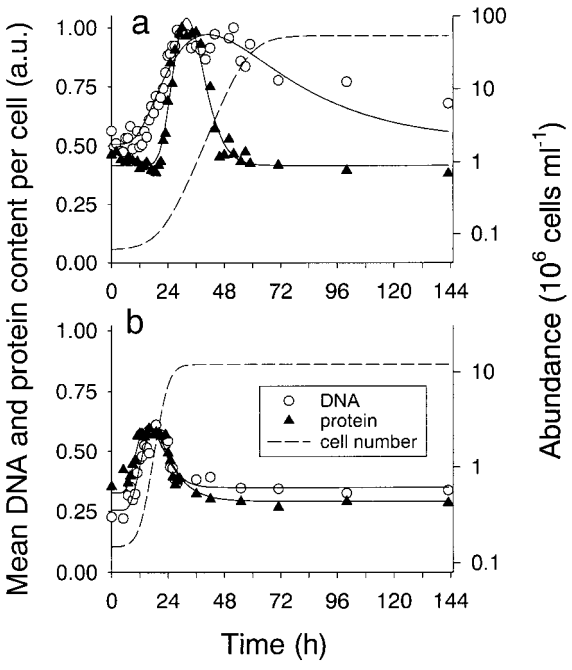

FIG. 3. Mean per-cell DNA and protein content of *Oceanospirillum* sp. (a) and *Pseudoalteromonas* sp. (b) during batch growth in pure cultures. a.u., arbitrary units.

contrast, rRNA fluorescence in *Oceanospirillum* sp. rapidly decreased to initial values at the onset of stationary phase.

DNA and protein fluorescence and cell sizes. Both organisms showed a bell-shaped curve of per-cell protein content during growth (Fig. 3). The relative protein fluorescence of *Oceanospirillum* sp. increased more rapidly than that of *Pseudoalteromonas* sp., to about 2.5 times of its initial value, whereas the maximum protein content of *Pseudoalteromonas* sp. was less than double its initial minimum. During stationary and exponential growth phases, the cellular protein content of *Oceanospirillum* sp. ranged from 75 (±15) fg $cell^{-1}$ to 164 (±6) fg $cell^{-1}$ and that of *Pseudoalteromonas* sp. ranged from 53 (±8) to 98 (±2) fg $cell^{-1}$, respectively. Maximum protein content per cell during mid-logarithmic growth corresponded with maximal cell volumes determined from size measurements of hybridized cells (data not shown). Mean per-cell DNA fluorescence intensity of both organisms approximately doubled during growth (Fig. 3). Except during mid-logarithmic growth, two subpopulations with different DNA content could be readily distinguished in both strains (Fig. 4). The high-DNA fraction represented about 25% in the *Oceanospirillum* sp. population even during stationary phase, whereas in *Pseudoalteromonas* sp., the high-DNA subpopulation declined to less than 5% in stationary-phase cells.

Population heterogeneity of *Oceanospirillum* sp. After 1 week of starvation and during lag phase, two distinct cell types were present in the *Oceanospirillum* sp. population: a small, nonmotile rod and a rare, large, fast-moving spirillum. The latter formed 1 to 2% of all cells at the time of inoculation and was not apparent during exponential growth or the first 24 h of stationary phase. FISH with a probe specific for *Oceanospirillum* sp. confirmed the purity of the culture (data not shown).

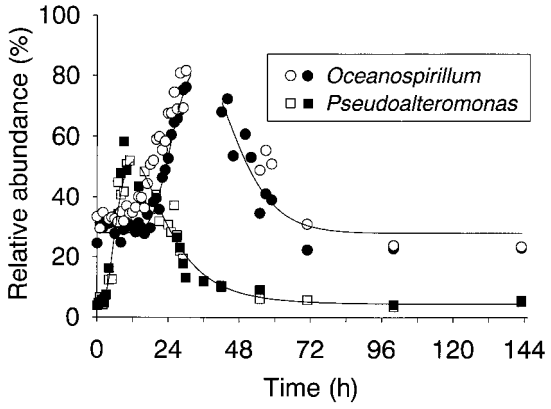

FIG. 4. Relative contribution of the fraction of cells with a high DNA content (multiple genome copies) in *Oceanospirillum* sp. and *Pseudoalteromonas* sp. The break in the curve indicates the time period during mid-logarithmic growth where a clear distinction of two DNA brightness classes was not possible.

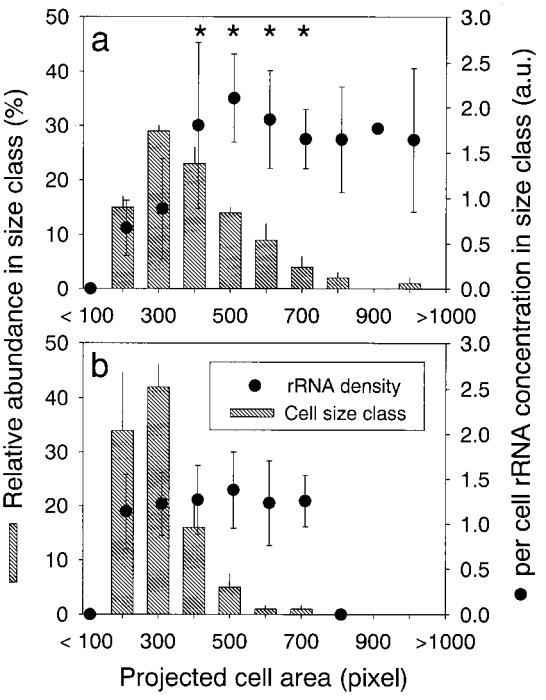

FIG. 5. Cell size distributions (bars) and distribution of mean 16S rRNA fluorescence (symbols) in different cell size classes (mean ± 1 standard deviation) at the end of lag phase of *Oceanospirillum* sp. (t = 16 h) (a) and of *Pseudoalteromonas* sp. (t = 8 h) (b). Asterisks indicate significant differences between the rRNA brightness of a size class and the brightness in the size classes of 100 to 200 and/or 200 to 300 pixels (analysis of variance, $P < 0.05$). a.u., arbitrary units.

The two subpopulations differed both in their cell sizes and mean rRNA fluorescence intensity, and the large size classes exhibited significantly higher rRNA concentrations at the end of lag phase (Fig. 5a) (analysis of variance, Scheffé post hoc comparisons, $P < 0.05$). No such subpopulations were observed in *Pseudoalteromonas* sp. (Fig. 5b). Two classes of cells with distinct protein content were distinguished in the DNA-rich subpopulation of *Oceanospirillum* sp. during lag phase (Fig. 6a to c) but not in *Pseudoalteromonas* sp. The *Oceanospirillum* sp. subpopulation of DNA-rich cells with distinctively higher protein fluorescence increased from <2% to 14% ± 2% after substrate addition and constituted >50% after the first doubling.

Competition between *Pseudoalteromonas* sp. and *Oceanospirillum* sp. Cocultures of the two strains always reached lower total cell densities ([5.8 ± 1.1] × 10^6 cells ml^{-1} [mean ± standard deviation]; $n = 6$) than the pure cultures of either strain. During coculture, *Oceanospirillum* sp. and *Pseudoalteromonas* sp. reached 7.5 and 25% of their pure culture maximum abundances, respectively (Fig. 7). The length of the lag phases and the duration of exponential growth of both organisms were similar in cocultures and in pure batch cultures. Thus, *Pseudoalteromonas* sp. had already ceased cell division at the onset of growth of *Oceanospirillum* sp. (Fig. 7). In both the batch and extended batch cocultures, where portions of substrates were added at intervals, *Oceanospirillum* sp. reached higher maximal cell densities than *Pseudoalteromonas* sp. During extended batch growth, *Pseudoalteromonas* sp. entered stationary phase when 30% of the total substrate had been added to the medium. It reached only 40% of the cell numbers attained in the batch cocultures (Fig. 7b). Total cell counts of *Oceanospirillum* sp. were similar after 100 h in both treatments. This resulted in three- to six-times-higher maximal densities of *Oceanospirillum* sp. than of *Pseudoalteromonas* sp. during extended batch cultivation, whereas the ratio of *Oceanospirillum* sp. to *Pseudoalteromonas* sp. was 1.3 in batch cocultures. No significant growth was observed in cocultures without substrate addition (data not shown).

DISCUSSION

Facultative eutrophic bacteria. Marine bacteria are frequently categorized into oligotrophic and eutrophic species. The latter are described as readily culturable, rare in bacterioplankton and prone to increase substantially in cell volume upon addition of substrate (40). The eutrophic bacterial strategy may represent the dominant type in some habitats, e.g., brackish waters (30), but common eutrophic isolates were generally rare in North Sea bacterioplankton (9). According to the above definition, both *Pseudoalteromonas* sp. and *Oceanospirillum* sp. are eutrophic marine genera.

Although frequently isolated, bacteria affiliating with the genus *Pseudoalteromonas* sp. were only occasionally detected on particles in coastal North Sea waters (8). High numbers of *Pseudoalteromonas*-specific viruses have been observed in fish feces (A. Wichels, personal communication), and a number of species from this genus are known to be associated with metazoans (18). Growth features that are commonly attributed to the opportunistic bacterial strategists were clearly more pronounced in the studied *Pseudoalteromonas* strain, such as the shorter lag phase upon transfer to fresh medium, a higher maximal growth rate, and lower total cell production (Fig. 1). It should be noticed, however, that all these parameters are potentially influenced by the composition of the cultivation medium. Thus, it would be premature to draw general conclusions about the ecological role of the two genera in North Sea coastal waters. Nevertheless, our results provide a model for

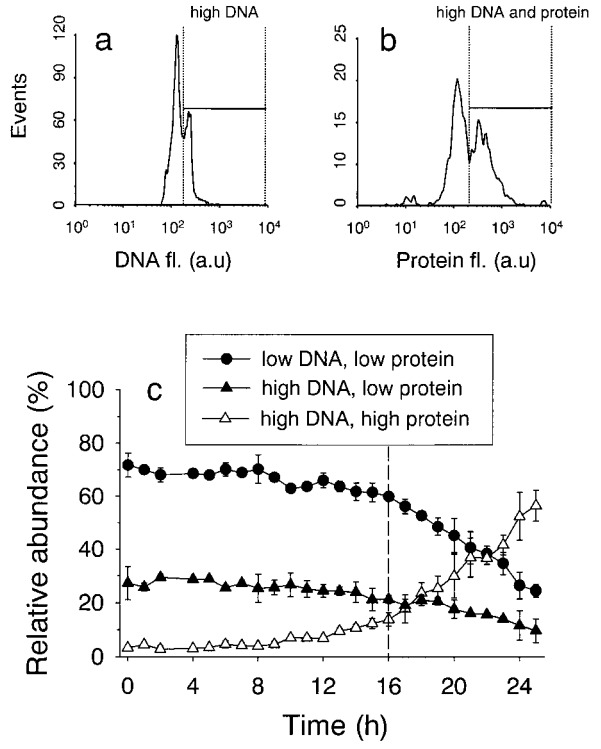

FIG. 6. (a) Histogram of bimodal distribution of DNA fluorescence (fl.) in *Oceanospirillum* sp. at *t* 16 h. (b) Histogram of bimodal protein fluorescence (fl.) within the high-DNA fraction of DNA fluorescence in panel a. (c) Relative abundances of subpopulations with different DNA and protein content in *Oceanospirillum* sp. during lag phase (0 to 16 h) and until the first doubling (25 h). a.u., arbitrary units.

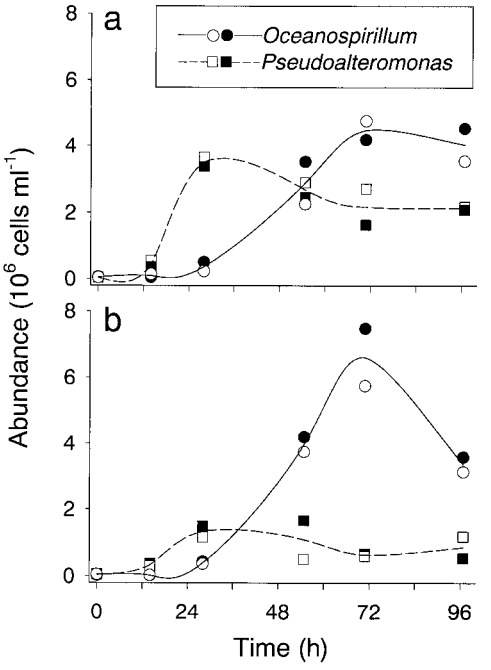

FIG. 7. Growth of *Oceanospirillum* sp. and *Pseudoalteromonas* sp. in cocultivation experiments. (a) Batch incubations. (b) Extended batch incubations with gradual substrate addition during 100 h.

understanding the outcome of our previous enrichment experiments on the same substrate mix (8) and illustrate the potential effects of substrate gradients on a two-species coculture system.

The stationary-phase subpopulation with a high DNA content in *Pseudoalteromonas* sp. was significantly smaller (Mann-Whitney U test, $P < 0.001$) than in *Oceanospirillum* sp., where it comprised roughly 25% of all cells (Fig. 4). Two other marine isolates also maintained large DNA-rich subpopulations in pure culture even during extended periods of starvation (20, 24). This contrasts somewhat with the view that the fraction of bacteria with a high DNA content found in pelagic microbial assemblages is representative of the growing part of the community (14, 25). Presently we can only speculate if and how the size of the high-DNA fraction during nongrowth is related to cultivation conditions or to the growth strategy of a population.

Marine spirilla have been known for several decades both from cultivation (44) and in situ observations (19). The phylogenetically closest relative of *Oceanospirillum* sp. strain KT0923, *O. commune*, was isolated from tropical surface waters (4). In coastal North Sea plankton, free-living bacteria related to *Oceanospirillum* sp. could be visualized in low densities (5×10^3 cells ml^{-1}) by FISH (8). The genus apparently includes culturable strains that are also present in the bacterioplankton and that are not oligotrophic by current definition (40).

A second phenotype was present in starved *Oceanospirillum* sp. cultures, which was clearly separated from the majority of cells by size, higher protein content, motility, and per-cell rRNA concentration (Fig. 5a and 6c). The rapid increase of such cells in stationary *Oceanospirillum* sp. after substrate addition (Fig. 6) suggests that cell multiplication mainly originated from within this subpopulation. Such heterogeneous growth has been observed before in marine bacteria. Upon substrate resupply, only a small fraction of a nongrowing *Vibrio* sp. population regained motility prior to cell multiplication (42). The starvation-induced motile subpopulation in *Oceanospirillum* sp. might thus be part of a more complex life strategy and, e.g., play a role in the colonization of new substrate patches (6).

Quantification of FISH staining intensities. Quantitative measurements of fluorescence intensities after FISH staining and image-analyzed microscopy yield two parameters as a potential measure of the 16S rRNA content per cell, the mean object gray value (optical brightness [O.B.]) and the total object gray value (integrated optical brightness [I.O.B.]). I.O.B. is the sum of fluorescence intensities of every positive pixel of a digitized image of a cell. The O.B. is the I.O.B. divided by the number of positive pixels, i.e., the mean pixel intensity.

The total amount of rRNA per cell that can be determined in chemical assays (22, 23), slot blot hybridizations (28), or flow cytometry evaluation of FISH-stained cells (5) is proportional to the sum of ribosomes per cell and is therefore equivalent to the I.O.B. of a hybridized cell. In batch culture studies, I.O.B. might be of limited use, because the bacterial cell volume substantially influences the total amount of ribosomes per cell. Thus, fluctuations in I.O.B. will to a large extent reflect changes in cell volume (35), even though the mean cell size and

I.O.B. are not expected to change completely in parallel during batch growth.

The O.B., on the other hand, is related to rRNA concentration, i.e., the density of ribosomes per unit of cell volume. The inherent advantage of the mean cell fluorescence as a measure of growth or protein synthesis potential is therefore its independence of changes in cell volume. It has been demonstrated that the cellular ribosome concentration (or its equivalent, the I.O.B. divided by the cell volume) increases with growth rate both in *Desulfovibrio vulgaris* and in *Pseudomonas putida* during balanced growth (26, 34).

The two bacterial strains studied clearly differed in their patterns of cellular 16S rRNA concentration during the various phases of their growth cycle (Fig. 5). During 100 h of stationary phase, high ribosome concentrations per cell were observed in *Pseudoalteromonas* sp. (Fig. 2b). Such maintenance of excess rRNA in a marine *Vibrio* sp. during starvation has been interpreted as an adaptation to a feast-and-famine existence, to allow rapid initiation of protein synthesis upon substrate resupply (12). The more rapid growth response of *Pseudoalteromonas* sp. both in pure culture and in cocultures (Fig. 7) and its selective enrichment in substrate-amended plankton samples (Table 1) provide evidence for this hypothesis. In contrast, the ribosome concentration of *Oceanospirillum* sp. declined upon the onset of stationary phase to the levels of the prestarved culture. The per-cell rRNA content of a *Sphingomonas* sp. that is thought to be representative of the free-living marine bacteria decreased by 90% upon cessation of growth (10). This development of cellular 16S rRNA concentrations during batch cultivation agrees with earlier findings that starvation periods of several weeks result in a much more pronounced decline of FISH detectability in cultures of *Oceanospirillum* sp. than in those of *Pseudoalteromonas* sp. (8) (Table 1).

We must, however, caution against overinterpretetion of the observed differences in ribosome content between the strains. A higher measurement frequency might be required to gain a detailed understanding of the actual development of cellular rRNA content during periods of rapid change, e.g., logarithmic growth. More studies are required to investigate other aspects which could potentially affect the patterns of rRNA concentration during batch growth. For example, it is presently unknown if and how the composition of the cultivation medium affects the patterns of macromolecular content. We used an artificial seawater mix that was specifically developed for the isolation of an oligocarbophilic marine *Sphingomonas* sp. and for subsequent ecophysiological investigations (38, 39), and this artificial seawater was successfully used for the isolation of the two studied strains. Yet this does not prove that the medium provided optimal growth conditions for the studied microbes.

Growth in cocultures. Numerous bacteria, including several *Pseudoalteromonas* species, are known to inhibit other microorganisms by releasing allelopathic substances (18). We found no indication for such interactions between the studied strains. Cell densities of *Oceanospirillum* sp. decreased during the first 24 h of nongrowth in the gradual enrichment, but no such decline was observed during batch cocultures, at higher total densities of *Pseudoalteromonas* sp. In contrast, mortality of stationary-phase *Pseudoalteromonas* sp. was higher in common batch culture enrichments. The lower abundances of both populations added together, compared to the density of either strain in pure culture (Fig. 1 and 7), rather indicated that cocultivation negatively affected the growth of both species.

Cocultivation and enrichment mode clearly influenced the growth rates and total cell production of the two species, but the duration of both the lag and of the respective exponential growth phases was unaffected by the treatments (Fig. 7). This may allow predictions about the performance of particular strains in batch coculture from parameters that can be readily determined in pure culture studies, provided that cocultivation is performed on the same medium.

From the length of the lag phases and the total cell production in pure cultures, it was predicted that the abundance ratio of the two strains in stationary-phase cocultures should be influenced by the mode of substrate addition. We hypothesized that *Pseudoalteromonas* sp. should dominate in a classic batch enrichment, whereas the more slowly but more "efficiently" growing *Oceanospirillum* sp. (Fig. 1) should be favored in a setup with gradually added substrates.

This was only partially verified. In batch cocultures the total cell production of *Oceanospirillum* sp. was indeed reduced to a much greater extent than that of *Pseudoalteromonas* sp., compared to pure cultures (Fig. 1 and 7a). The most obvious advantage of *Pseudoalteromonas* sp. under these conditions was the shorter growth delay upon substrate addition, in both pure and mixed cultures (Fig. 1 and 7). Therefore, *Pseudoalteromonas* sp. probably consumed the bulk of available organic matter. On the other hand, *Oceanospirillum* sp. was not only capable of growth on the fraction of substrate that was not consumed by *Pseudoalteromonas* sp.; it eventually even reached higher total densities than the other strain in batch coculture. This agrees with the higher total cell production of *Oceanospirillum* sp. in pure culture (Fig. 1).

In contrast, the shorter lag phase of *Pseudoalteromonas* sp. would represent no specific advantage during gradual substrate addition. The significantly reduced growth of *Pseudoalteromonas* sp. in extended batch cocultures (Mann-Whitney U test, $n = 8$, $P < 0.01$) (Fig. 7b) is therefore most likely the consequence of a lower amount of available substrate at the onset of cell multiplication. Less than 20% of the organic carbon of the batch culture had been added at that time point. The gradual addition of substrates to stationary cocultures of the two strains did not result in lower final abundances of *Oceanospirillum* sp., and the slopes of cell increase during exponential growth of *Oceanospirillum* sp. were unaffected or even slightly higher in the gradual enrichments (Fig. 7a and b). In summary, there is evidence for both strains that the mode of substrate addition affected competition between *Pseudoalteromonas* sp. and *Oceanospirillum* sp. in batch coculture.

Conclusions. Under our specific cultivation conditions, neither the length of lag phases of the studied strains nor the duration of logarithmic growth appeared to be affected by cocultivation. The selective enrichment of *Pseudoalteromonas* sp. on a particular substrate mix, as previously observed in pelagic samples (8), is therefore most likely related to a shorter growth delay upon addition of these substrates. The *Pseudoalteromonas* sp. strain, moreover, maintained high stationary-phase levels of cellular rRNA, which has been predicted for marine bacteria with a more opportunistic life strat-

egy. This hypothesis was supported by the outcome of gradual substrate addition to cocultures, which resulted in a shift of total cell production towards *Oceanospirillum* sp. Gradual enrichment might, therefore, provide a tool for the directed isolation of bacteria that are otherwise rapidly overgrown.

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