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Functional characteristics of culturable bacterioplankton from marine and estuarine environments

Summary. Information on the structure of bacterioplankton communities is continuously increasing, while knowledge of their metabolic capabilities remains limited. In this study, the metabolic capacity of bacterioplankton was investigated, as such information is necessary to fully understand carbon cycling and other biogeochemical processes. The diversity of dominant culturable chemoorganotrophic bacteria from one estuarine and three marine environments was analyzed by random isolation of colony-forming units on solid media, taxonomical identification by partial 16S rRNA gene sequence analysis, and functional characterization of the isolates. A total of 76 16S rRNA gene sequences, representing 19 different genotypes, were obtained from the four sampling localities, including *Bacillus*, *Pseudomonas*, *Pseudoalteromonas*, *Vibrio*, and *Erythrobacter* as the most frequently isolated genera. The range of metabolic functions possessed by the cultured bacterial assemblages differed significantly between sites. Similarly, the percentage at each sampling station of bacteria capable of performing a specific function was significantly different for 18 of the 25 investigated metabolic functions. At two localities, the bacterial assemblages were dominated by a single genus (*Pseudoalteromonas* or *Erythrobacter*) and appeared to be functionally specialized. More than 95% of the isolates were capable of utilizing dissolved free amino acids and protein as their sole nitrogen sources, and all isolates of the specialized assemblages expressed β-glucosidase. Furthermore, only some of the isolates were able to utilize $NH4^+$, while up to two thirds of the isolates of the two marine sites were able to grow on NO_3^-. [**Int Microbiol** 2004; 7(3):219–227]

Key words: culturable bacterioplankton · physiological characterization · taxonomic and functional diversity · metabolic capacity

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Introduction

Bacterioplankton plays a key role in the turnover of carbon and nitrogen in aquatic environments. Recent studies on the genetic diversity of bacterioplankton communities have shown taxonomic variation in both time and space [3,14]. However, since this information is based primarily on 16S rRNA gene sequences, the physiological characteristics and the ecological roles of the identified genotypes are largely unknown. Most of the currently available knowledge on bacterioplankton community function derives from “black box” studies in which turnover of dissolved organic matter by uncharacterized bacterial communities is measured [33], i.e. the role of the individual community member is unknown. A few studies, however, have tried to attribute specific functions to specific phylogenetic groups. For instance, Cottrell and Kirchman [10,11] showed, by combined microautoradiogra-

phy and fluorescence in situ hybridization (FISH), that taxonomic groups at the division and subclass levels differed in their potential for utilizing organic compounds, and Davey et al. [13] demonstrated that differences in enzyme activity between communities could be explained by differences in species composition. Similarly, Cho and Giovannoni [8], in a study of the growth characteristics of marine oligotrophic gamma-proteobacteria under different culture conditions (temperature and carbon concentration), found that the physiological properties generally followed the phylogenetic relationships.

The aim of the present study was to assist in filling the knowledge gap between identity and function, i.e. to address the fundamental question of which functions are attributable to which species (genotype) in the natural environment. Hence, we carried out a comparative investigation of the relationship between the taxonomic and functional diversity (metabolic capacity) of bacterioplankton at the species level. The focus of the study was the culturable fraction of the bacterioplankton since a thorough physiological characterization requires bacteria in axenic culture [2,50]. Culture-dependent techniques (both traditional [6,26,32,40] and new high-throughput culturing methods [8,46]) have experienced a revival recently, and new evidence suggests that culture-independent and culture-dependent methods identify unique subsets of bacterial species [17,32,40]. Bacterial species potentially identified by culture-independent methods were, therefore, most likely overlooked by our approach. However, because the culturable fraction appears to represent the active populations [18,41,47], the isolated bacteria probably were of ecological significance at the time of sampling.

Materials and methods

Sampling. Samples of Danish marine and estuarine water were collected at a depth of 3.5 m in the Kattegat, at 30 m (chlorophyll maximum) and 79 m in the Skagerrak, and at 1 m in the shallow estuary of Roskilde Fjord. Coordinates and characteristics of the sampling stations are listed in Table 1.

Chemical analyses. Water samples for analysis of o-phosphate, total dissolved nitrogen (TDN), nitrate, urea, dissolved free amino acids (DFAA), and dissolved combined amino acids (DCAA) were filtered through 0.2-µm cellulose acetate filters (Sartorius) and stored in acid-rinsed bottles at –18ºC. TDN was measured on a Dohrman TN1900 nitrogen analyzer using urea as standard. Nitrate, *o*-phosphate, and urea were determined on an AlpKem Flowsolution IV autoanalyzer according to procedures of the manufacturer, except urea, for which the method by Price and Harrison [44] was adopted. Concentrations of DFAA and DCAA (water samples hydrolyzed according to Jørgensen and Jensen [29]) were determined as fluorescent *o*-phthaldialdehyde derivates by HPLC according to the method of Jørgensen et al. [27].

For analysis of dissolved organic carbon (DOC), 0.2-µm cellulose-acetate-filtered samples were stored in closed precombusted glass sample vials (Shimadzu) at –18ºC. DOC was measured on a Shimadzu TOC 5000 analyzer (oxidation at 680ºC, Pt-covered aluminum oxide as a catalyst). At least triplicate injections were made per sample. Acidified solutions (Suprapur HCl, Merck, Darmstadt, Germany) of potassium biphthalate in Milli-Q water were used as standards.

Counting and isolation of bacteria. Samples for bacterial total counts were preserved with formaldehyde 2% (v/v) final concentration for Roskilde Fjord and 3.7% (v/v) final concentration for Skagerrak and Kattegat). Bacteria were counted under epifluorescence microscopy using acridine orange staining (Roskilde) or 4;6-diamidino-2-phenylindole (DAPI) staining (Skagerrak and Kattegat) according to the methods of Hobbie et al. [24] and Porter and Feig [43], respectively.

The number of colony-forming units (CFU) was determined by plate-spreading dilutions of filtered water (0.8-µm pore-size, Nuclepore polycarbonate filter) on 10% nutrient strength ZoBell agar plates [57]. The medium was diluted in order to reduce substrate-accelerated death. Plates contained 0.5 g Bacto peptone (Difco, Detroit, MI), 0.1 g yeast extract (Difco), 0.015 g $FePO_4$, $4H_2O$ (Riedel-de Haën AG, Seelze-Hannover, Germany) and 15 g agar (Difco) per liter of water from the appropriate sampling locality. The

Table 1. Characteristics of sampling stations. CFU, colony-forming units; DOC, dissolved organic carbon, TDN, total dissolved nitrogen; DCAA, dissolved combined amino acids; DFAA, dissolved free amino acids

Characteristics	Skagerrak (30 m)	Kattegat	Skagerrak (79 m)	Roskilde Fjord
Location	57º 96´ N; 10º 78´ E	57º 63´ N; 11º 07´ E	57º 84´ N; 10º50´E	55º 43´ N; 12º 04´ E
Sampling depth	30 m (chl *a* max)	3.5 m	79 m	1 m
Sampling date	17 July 1997	17 July 1997	19 July 1997	24 June 1997
Salinity (g/l)	35	25	35	17
Temperature (ºC)	8.9	18.4	8.1	17.3
Total counts (cells/ml)	8.91×10^5	6.41×10^5	5.75×10^5	7.24×10^5
CFU (colonies/ml)	4.13×10^2	6.07×10^2	3.56×10^2	8.83×10^3
	(5.8×10^1)[a]	(2.8×10^1)	(8.9×10^1)	(1.1×10^3)
DOC (mg/l)	2.48 (0.5)	3.04 (0.2)	1.27 (0.1)	7.89 (0.8)
PO_4 (µM)	0.61 (0.03)	0.52 (0.02)	0.61 (0.13)	2.83 (0.12)
TDN (µM-N)	5.6 (2.3)	11.2 (1.9)	9.4 (2.6)	40.1 (3.2)
DCAA (nM-N)	582.2 (195)	1049.5 (389)	776.6 (255)	2995 (556)
DFAA (nM-N)	115.5 (17)	137.7 (40)	137.3 (27)	259 (76)
Urea (µM-N)	0.29 (0.06)	0.40 (0.08)	1.37 (0.02)	0.84 (0.24)
NO_3^- (µM-N)	0.52 (0.3)	0.28 (0.1)	0.52 (0.34)	1.49 (0.31)

[a] Standard deviations are shown in parentheses.

water had been sequentially filtered through 1.0- and 0.2-µm-pore-size filter cartridges (Millipore Corp., Bedford, MA), and a 0.2-µm GV filter (Nuclepore Corp., Pleasanton, CA). To insure that slow-growing strains were not overlooked, plates were incubated for 11 days at 16ºC (marine stations) or at room temperature (Roskilde Fjord). The incubation time of 11 days was based on a preliminary experiment (not shown) demonstrating that numbers of colonies appearing on the agar plates reached a constant value after 11–12 days.

Dilutions resulting in plates with ca. 30–50 colonies were preferentially used to isolate 30 colonies per sampling station. Randomness in colony selection was assured by observing agar plates in an orientation determined before the appearance of colonies, and by choosing colonies in a consistent manner, using horizontal gridlines. Rather than choosing different colonies, to obtain an estimate of species richness, the isolation procedure employed selected for abundant culturable bacteria and provided an estimate of their relative abundances. Most colonies were white, round and smooth, and a total of 16 different colony morphologies were observed. Isolates were stored frozen at –80ºC in 50% (v/v) glycerol in liquid 10% nutrient strength ZoBell medium.

Characterization of isolated bacteria. Isolated bacteria were grouped on the basis of their morphological and physiological characteristics. Isolates having the same colony morphology were often different with respect to their physiological characteristics. Gram reaction was determined by lysis with 3% (w/v) KOH [21]. Catalase activity was detected by the presence of bubbles after addition of one drop of 3% (v/v) H_2O_2 to colonies. Cytochrome oxidase activity tests were considered positive when cells formed blue pigments after being streaked on filter paper wetted with 1% (w/v) of N:N:N:N-tetramethyl-*p*-phenyl-diaminedihydrochloride. NO_3^--reduction, indole production from tryptophan, fermentation of glucose, L-arginine dihydrolase activity, urease activity, hydrolysis of gelatin, and growth on 12 different organic carbon compounds were determined using the API 20NE test system (bioMérieux, Marcy-l'Etoile, France) as recommended by the manufacturer.

The potential to grow on specific N sources was also assessed. Cells were grown for 2 days in liquid 10% strength ZoBell medium of appropriate salinity and starved for 4 days at 20ºC in sea salt (Sigma) or minimal medium [23] without an added carbon and nitrogen source. The starvation period was introduced in order to avoid positive signals (subsequent growth) resulting from utilization of internal carbon and nitrogen reserves. Following starvation, growth was tested in microtiter wells (Nunc, Naperville, IL) with 1 mM nitrogen derived from one of the following compounds as the sole nitrogen source: NH_4Cl (Merck), $NaNO_3^-$ (Merck), urea (Sigma, St. Louis, MO), a mixture of 22 amino acids (alanine, arginine hydrochloride, aspargine, aspartate, cysteine, cystine, glutamate, glutamine, glycine, histidine hydrochloride, trans-4-hydroxyproline, isoleucine, leucine, lysine hydrochloride, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine) (Sigma), casein (Sigma) or *Escherichia coli* DNA (Sigma). The nitrogen compounds were dissolved either in sea salt amended with 1 mM phosphate derived from KH_2PO_4 (Merck) and 100 mM carbon from D-glucose, H_2O (Merck), or in minimal medium with glucose. The applied nutrient concentrations were chosen so that growth could be measured as an increase in optical density (OD_{590}) on an ELISA reader EL314 (Bio-Tek instruments, Winooski, VT). Wells with nitrogen but no cells, and wells with cells but no nitrogen served as controls. Growth was followed for 5 days. Glassware and pipette tips were acid rinsed to avoid contamination with nitrogen. An isolate was considered capable of growth on a specific nitrogen source if growth occurred in at least two out of three or three out of four replicate tests. From each locality, 28 to 29 of the isolates were characterized with respect to enzymatic activities and carbon assimilation (API 20NE test system), while 18 to 22 isolates were characterized with respect to nitrogen assimilation in the microtiter wells. The remaining isolates failed to resume growth after storage.

16S rDNA sequencing. DNA was extracted by transferring colonies with a sterile toothpick into 1.5-ml safe-lock Eppendorf tubes containing 200 µl 0.04 M Tris-acetate EDTA buffer (Sigma). Tubes were vortexed, boiled for 10 min, left at room temperature for 10 min, and centrifuged (15,000 g, 10 min, 4ºC). Supernatants containing the DNA were then transferred to new tubes and stored at 5ºC.

DNA coding for 16S rRNA was amplified by polymerase chain reaction (PCR) (10 min at 95ºC; 35 cycles of 30 s at 95ºC, 30 s at 55ºC, 2 min at 72ºC; 6 min at 72ºC; final cooling at 4ºC). The PCR protocol of Johnsen et al. [25] was applied, except that (i) Ampli Taq Gold polymerase (Applied Biosystems, Foster City, CA.), and (ii) primers F1 at position 9–27 and R13 at position 1525–1542 [15] were used. After PCR, products were purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. A partial sequence of the 16S rDNA was obtained by sequencing (ABI PRISM 3700 DNA Analyzer, BigDye Terminator, Applied Biosystems) the PCR product in both directions using primer F1 and a primer (CGTATTACCGCGGCTGCT) modified after the method of Lane et al. [34] and binding at position 518–535. Sequences were aligned against those from the Ribosomal Database Project II [35].

Approximately two-thirds of the 30 strains from each sampling station were sequenced. The remaining isolates either were unable to grow after long-term storage or failed in producing a PCR product or a 16S rDNA sequence.

Accession numbers. Nucleotide sequences were filed in GenBank under the following accession numbers: A5: AF293089; A6: AF293090; A7: AF325137; A10: AF325138; A22: AF325139; A25: AF325135; A28: AF293091; B3: AF293092; B4: AF293093; B20: AF325140; B25: AF325141; B30: AF325142; D1: AF293094; D3: AF325143; D6: AF325144; D15: AF325146; D23: AF325147; D25: AF293095; D30: AF325148; F15: AF325136; F16: AF325149; F19: AF325150. Identical clone sequences were not filed.

Statistical analysis. The ability of a bacterial isolate to perform a specific metabolic function (e.g. ability to grow on casein) was treated as a binary response, i.e. the population of bacterial isolates being able to perform a metabolic function was binomially distributed with probability parameter "p" and sample size "n". The data were analyzed using logistic regression under the framework of generalized linear models [37]. A likelihood-ratio test was used to determine whether the frequency of an individual metabolic function, or the frequencies of all metabolic functions combined, were significantly different between sampling stations. Models applied were $N_{positive}/N_{total}$ = station and $N_{positive}/N_{total}$ = $station_i$ + $functional\ group_j$ + $station_i$ × $functional\ group_j$ + $function_n$($functional\ group_j$), respectively. The logistic regressions were carried out using PROC GENMOD(SAS/STAT). A χ^2 test was used to test whether the total numbers of functions present at each station were significantly different. A phenogram (dendrogram) based on the characterization with respect to enzymatic capabilities and growth on various carbon and nitrogen sources was constructed using the Treecon software [53]. A bootstrap analysis with 500 sample replications was performed using the simple matching similarity index and the UPGMA algorithm.

Results

Diversity of isolated bacteria. A total of 76 partial 16S rRNA gene sequences (genotypes) were obtained from the four sampling sites. Of these, 19 were found to be different (Table 2). In three cases, identical sequences were obtained at different sampling stations: Isolate B4 was identical to D3, A28 was identical to B20, and A25 was identical

Table 2. Diversity of isolated bacteria

Isolate[a]	Locality	No[b]	Nearest phylogenetic neighbour(s)	Similarity (%)	Sampling locality of neighbor(s)	Reference
A6	Skagerrak (30 m)	7	*Bacillus* sp. str. 47083	100	Environmental sample, France	[16]
A5, A7	Skagerrak (30 m)	1,1	*Vibrio* sp. SCB30	>99	Scripps Pier (California)	[42]
			Vibrio sp. E-twt2.M7p-b	>99	*Ridgeia piscesae* Pacific Ocean	[31]
A10	Skagerrak (30 m)	2	*Brevundimonas* sp. BAL3	>99	Baltic Sea	[41]
			Erythrobacter (*citreus*)	>99	Mediterranean Sea (0.2 µm filtrate)	[55]
A22	Skagerrak (30 m)	1	*Brevundimonas* sp. V4.BO.10	>99	Mediterranean Sea (150 m depth)	[1]
			Brevundimonas sp. BAL51	>99	Baltic Sea	[22]
			Pseudomonas sp. BAL16	>99	Baltic Sea	[22]
A25 (=B3), A28 (=B20)	Skagerrak (30 m)	3,2	*Pseudomonas* sp. CR-FL29	>99	Columbia River	[12]
			Pseudomonas sp. OA146	>99	Baltic Sea (osmolyte producer)	[38]
			Pseudomonas sp. BAL16	>99	Baltic Sea	[22]
B3 (=A25), B20 (=A28)	Kattegat	3,13	Same as A25 and A28			
B30	Kattegat	2	*Vibrio* sp. SCB30	>99	Scripps Pier (California)	[42]
B25	Kattegat	1	*Pseudomonas mandelii* CIP 10523	>99	French mineral water	[54]
B4 (=D3)	Kattegat	1	*Pseudoalteromonas haloplanktis* SKA19	100	Skagerrak	PH[c]
			Pseudoalteromonas haloplanktis IAM14160T	100		[20]
D1	Skagerrak (79 m)	1	*Pseudoalteromonas haloplanktis* SKA19	>99	Skagerrak	PH[c]
			Pseudoalteromonas haloplanktis IAM14160T	>99		[20]
D3 (=B4)	Skagerrak (79 m)	10	Same as B4			
D15	Skagerrak (79 m)	2	*Pseudoalteromonas* sp. SKA20	>99	Skagerrak	PH[c]
D23	Skagerrak (79 m)	2	*Pseudoalteromonas aurantia* ATCC 33046T	>97		[20]
D25	Skagerrak (79 m)	1	*Pseudoalteromonas* sp. NIBH P2M11	>99	Japan Trench	[36]
			Pseudoalteromonas sp. MS23	>99	Mediterranean Sea (marine snow)	[4]
D6	Skagerrak (79 m)	2	*Pseudoalteromonas* sp. P-1P41	100	Pacific Ocean (PAH degrader)	HS[d]
			Pseudoalteromonas distincta KMM638T	100		[48]
D30	Skagerrak (79 m)	2	CFB (*Tenacibaculum ovolyticum*)	>94		[51]
F15, F19	Roskilde	1,17	*Erythrobacter longus* Och101	>99	Japan and Australia, marine	[49]
F16	Roskilde	1	*Micrococcus* sp. AC-47	100	Belgium, mine	[5]

[a] Name of representative isolate. Names in parentheses indicate isolates from other localities having a 16S rDNA sequence (genotype) identical to that of the representative isolate.
[b] Number of isolates having a partial 16S rDNA sequence identical to that of the representative isolate.
[c] Pinhassi and Hagström (2000). Data from the Ribosomal Database Project II (RDP).
[d] Hedlund and Staley (2000). Data from the Ribosomal Database Project II (RDP).

to B3 (Table 2). Isolates with the B4/D3 sequence were most abundant at Skagerrak (79 m) but were also found in Kattegat (Fig. 1, Table 2). These isolates had a 100% partial 16S rDNA similarity with *Pseudoalteromonas haloplanktis* SKA19 previously isolated from the Skagerrak (Table 2). The two other pairs of isolates (A25/B3 and A28/B20) were found in the Skagerrak (30 m) and Kattegat (Fig. 1, Table 2) and were closely related to *Pseudomonas* sp. OA146, which is a salt-adapted pseudomonad isolated from the Baltic Sea that synthesizes glucosylglycerol as an osmolyte [38].

The number of different genotypes within each locality varied between three and seven (Fig. 1). At both Skagerrak sites, seven different 16S rRNA gene sequences were identified (Fig. 1). *Bacillus* spp. and pseudomonads dominated in the Skagerrak (30 m), constituting 41% and 29% of the sequenced isolates, respectively (Fig. 1, Table 2), while pseudoalteromonads made up 90% of the sequenced isolates at Skagerrak (79 m) (Fig. 1, Table 2). In the Kattegat, five different 16S rRNA gene sequences were obtained (Fig. 1), with *Pseudomonas* being the dominant genus (85%) (Fig. 1, Table 2). At the estuarine station in Roskilde Fjord, 18 out of 19 isolates were identified as *Erythrobacter* and only three different genotypes were found (Fig. 1, Table 2).

The 16S rRNA gene sequence similarity between 17 of the different isolates and previously reported sequences obtained from geographical distant localities was higher than 99% (Table 2). The two isolates (D23 and D30) that were less than 99% similar to literature sequences were both found in the Skagerrak (79 m). Isolate D30 has recently been described as a new species within the genus *Tenacibaculum* [19].

Functional characteristics of isolated bacteria. The number of metabolic functions possessed by the isolated assemblages of bacteria from the four sampling localities was

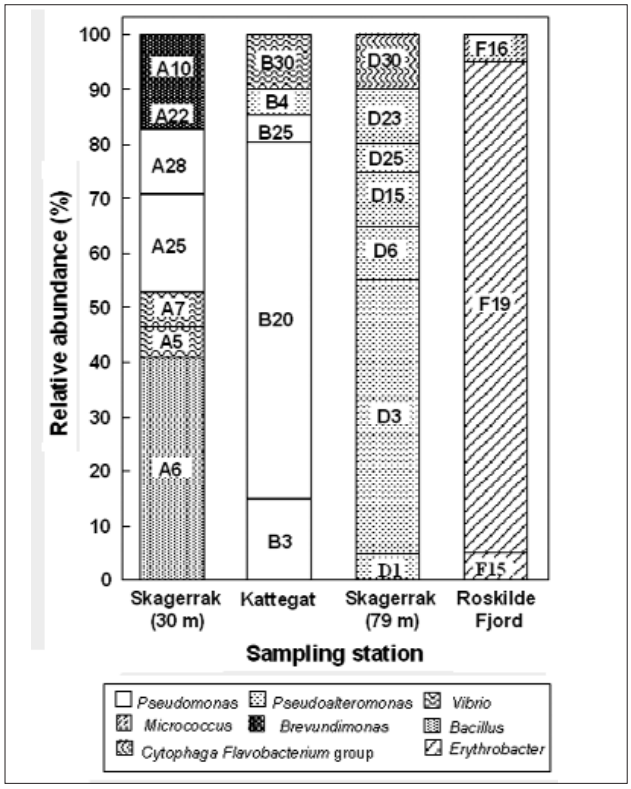

Fig. 1. Relative abundance of sequenced isolates at the four sampling stations. Letter-figure combinations refer to the groups of genotypes listed in Table 2.

significantly different ($p = 0.0025$). Similarly, the percentage at each sampling station of bacteria capable of performing a specific function differed significantly ($p < 0.05$) for 18 of the 25 investigated metabolic functions (Table 3).

The Kattegat and Skagerrak (30 m) isolates appeared to be more versatile than those of Roskilde Fjord and Skagerrak (79 m) as 88% and 85% of the investigated metabolic characteristics were expressed at these localities, respectively, while only 62% and 50% were found in Roskilde Fjord and Skagerrak (79 m), respectively. Furthermore, specific metabolic functions were expressed either by all or by none of the members of the Roskilde Fjord and Skagerrak (79 m) assemblages, whereas in Skagerrak (30 m) and Kattegat the ability to perform a metabolic function was more evenly shared among the isolates (Table 3). A cluster analysis (Fig. 2) based on the functional characteristics of the isolates demonstrated that the Roskilde Fjord and Skagerrak (79 m) assemblages clustered in relatively distinct groups (except for isolate F16), whereas no distinct functional clusters were observed for Kattegat and Skagerrak (30 m).

More than 95% of the isolates were capable of utilizing DFAA and protein as sole nitrogen sources, and at Skagerrak (79 m) and Roskilde Fjord all isolates expressed β-glucosidase activity (Table 3). Surprisingly, not all isolates were able

Table 3. Distribution and frequency of metabolic functions. All isolates were able to grow on dissolved free-amino acids, while none utilized adipate, phenyl-acetate and DNA

Functional character		Frequency (%) of functional characters within bacterial assemblages				p[b]
		Skagerrak (30 m)	Kattegat	Skagerrak (79 m)	Roskilde Fjord[a]	
Enzymatic activity	NO_3^- reduction	36	28	11	5	0.0125
	Tryptophanase	29	21	4	0	0.0018
	Fermentation of glucose	7	7	7	0	0.4146
	Arginine decarboxylase	25	59	4	0	<0.0001
	Urease	0	17	0	5	0.0096
	β-Glucosidase	46	17	100	100	<0.0001
	Protease (gelatinase)	61	41	71	5	<0.0001
Assimilation	D-Glucose	39	24	0	5	<0.0001
	L-Arabinose	7	17	0	5	0.0533
	D-Mannose	32	24	0	5	0.0004
	D-Mannitol	7	24	0	5	0.0080
	N-acetyl-D-glucosamine	32	24	0	5	0.0004
	Maltose	36	21	0	5	0.0002
	Gluconate	32	17	0	5	0.0008
	Caprate	25	66	0	0	<0.0001
	L-Malate	61	79	7	5	<0.0001
	Citrate	29	66	7	0	<0.0001
	NH_4^+	67	38	68	5	<0.0001
	NO_3^-	67	24	5	0	<0.0001
	Urea	17	14	0	0	0.0270
	Protein (casein)	94	100	95	100	0.3697

[a] All 5% values were due to isolate F16 (Table 2). [b] Probability (p) of equal frequency of function.

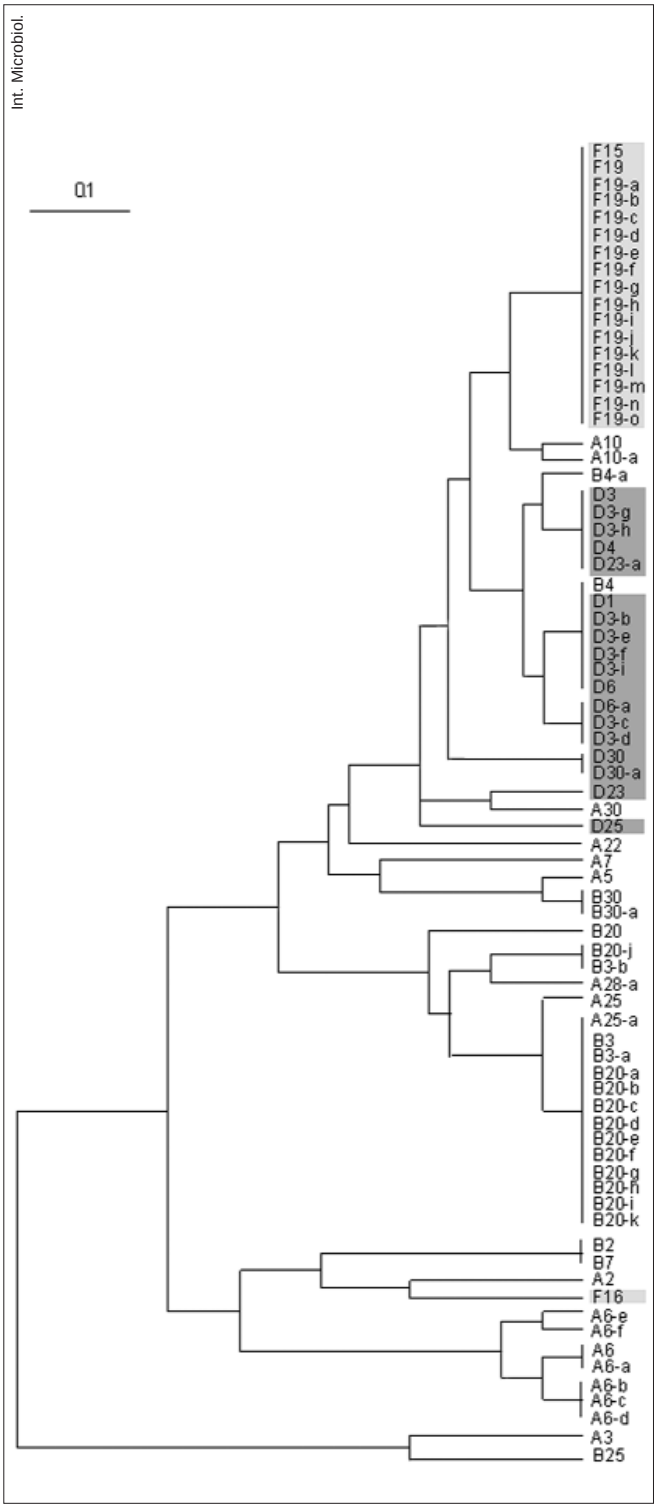

Fig. 2. Dendrogram showing the relationship of isolated strains on the basis of their physiological capacities (Table 3). Groups of isolates with identical 16S rDNA sequences are labeled with lower-case letters. Light gray shading, position of isolates from Roskilde Fjord in the dendrogram; dark gray shading, position of the Skagerak (79 m) isolates. Bar=10% divergence. Since the dendrogram was based on 25 physiological characters, bootstrap values were often low (range: 2–100%) compared to dendrograms based on the sequence of partial or total 16S rRNA genes.

to utilize NH_4^+ as sole N source, and two thirds of the isolates in Skagerrak (30 m) and about 40% of those in Kattegat were able to grow on NO_3^-. None of the isolates grew on DNA, adipate or phenyl acetate (Table 3).

Discussion

The taxonomy and potential metabolic capacity of culturable bacterioplankton from one estuarine and three marine localities were studied. The metabolic potential rather than the in situ activity of the isolates was determined since in situ measurements are only representative of the conditions at the time of sampling. Assessment of the potential metabolic capacity, therefore, provides a better picture of the ecological roles of the organisms. The bacterial isolates from the Skagerrak (79 m) were dominated by a single genus (*Pseudoalteromonas*) and were functionally less versatile than those in Kattegat and Skagerrak (30 m) (Fig. 1, Table 2). Consistent with this, all isolates tested positive for β-glucosidase activity, while only a few were able to grow on low-molecular-weight carbon substrates (Table 3). If the culturable bacteria represent those that are active in the environment, as suggested by Ellis et al. [18], Pinhassi et al. [41] and Rehnstam et al. [47], this suggests that the Skagerrak (79 m) community was adapted to growth on refractory carbon possibly in the form of aged organic matter. Roskilde Fjord isolates were almost completely dominated by a genotype closely related to *Erythrobacter longus* OCh101, which has been frequently isolated from eutrophic environments [49]. Similar to the isolates in Skagerrak (79 m), only a few of those from Roskilde Fjord were able to grow on low molecular weight substrates while they all showed β-glucosidase activity (Table 3), suggesting that the community of culturable bacteria was adapted to growth on refractory (allochthonous) carbon. *Bacillus* and *Pseudomonas* spp. dominated the Skagerrak (30 m) and Kattegat samples (Fig. 1, Table 2). The *Bacillus* isolates were capable of metabolizing a diverse range of carbohydrates. Similarly, pseudomonads have simple nutritional requirements and grow well in media containing organic matter in solution [39]. Thus, the bacteria at the chlorophyll *a* maximum in the Skagerrak (30 m) and in the surface water in Kattegat probably grew on labile dissolved organic matter produced by the phytoplankton [9].

All, or nearly all, of the isolated bacteria were able to utilize dissolved free and combined amino acids as their sole nitrogen sources (Table 3). In contrast, not all isolates were able to utilize NH_4^+. This was surprising since NH_4^+ is generally considered to be an important source of N for bacterioplankton [33]. Equally surprising was the fact that two thirds

of the isolates in Skagerrak (30 m) and about 40% of those in Kattegat were able to grow on NO_3^- (Table 3) since nitrate is not considered to be a major contributor to bacterial nitrogen requirements [56]. No isolates grew on DNA as the sole nitrogen source (Table 3) suggesting that it served primarily as a supplementary source of nitrogen [28] or phosphorus [52]. Also, studies have shown that bacterioplankton involved in cycling of dissolved DNA preferably take up small-size DNA fragments (100 bp) [27]. In the present study, the size of the DNA fragments might have been too large. Only a few of the bacterial isolates could grow on urea (Table 3). This is consistent with results in the literature; for example, Cho et al. [7] reported that bacterioplankton are significant urea producers but not decomposers, and Jørgensen et al. [30] found that urea was produced when amino acids were decomposed.

In order to advance our understanding of carbon cycling and other biogeochemical processes in aquatic ecosystems, detailed studies on the functional characteristics of bacterioplankton are needed. The results reported in this study expand the knowledge of the functional roles of marine pelagic bacteria. Currently, the best way to obtain in-depth information on growth characteristics and enzymatic capabilities at the species level is to study cultured bacteria. However, to overcome the potential biases of cultivation-dependent approaches, and to link measurements of potential metabolic capability with those of in situ activity, new methods, such as DNA/RNA-based stable-isotope probing [45], should be developed and applied in conjunction with classical ones.

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Características funcionales del bacterioplancton cultivable de ambientes marinos y de estuario

Resumen. La información sobre la estructura del bacterioplancton aumenta continuamente, mientras que el conocimiento de sus capacidades metabólicas sigue siendo limitado. En este estudio se investigó la capacidad metabólica del bacterioplancton, dado que dicha información es necesaria para comprender completamente el ciclo del carbono y otros procesos biogeoquímicos. La diversidad de las bacterias quimioorganotrofas cultivables que predominaban en un ambiente de estuario y en tres ambientes marinos se estudió aislando al azar unidades formadoras de colonias en medios sólidos, realizando la identificación taxonómica por medio del análisis de secuencias de genes del 16S rRNA, y mediante la caracterización funcional de los aislados. A partir de las cuatro localidades de muestreo se obtuvieron 76 secuencias de genes del 16S rRNA, que representaban 19 genotipos diferentes. Los géneros aislados con mayor frecuencia fueron *Bacillus*, *Pseudomonas*, *Pseudoalteromonas*, *Vibrio* y *Erythrobacter*. El margen de las funciones metabólicas que tenían los conjuntos (*assemblages*) de bacterias cultivadas difería notablemente entre las distintas localidades de muestreo. De manera similar, en cada estación de muestreo el porcentaje de bacterias que podían realizar alguna función específica era muy diferente para 18 de las 25 funciones metabólicas investigadas. En dos localidades predominaba un sólo género (*Pseudoalteromonas* o *Erythrobacter*) y parecían desempeñar funciones especializadas. Más del 95% de los aislados podían utilizar como única fuente de nitrógeno aminoácidos libres y proteínas disueltos, y todos los aislados de los conjuntos especializados expresaban β-glucosidasa. Además, sólo algunos de los aislados podían usar $NH4^+$, mientras que hasta un tercio de los aislados en las dos localidades marinas podían crecer con NO_3^-. [**Int Microbiol** 2004; **7**(3):219–227]

Palabras clave: bacterioplancton cultivable · caracterización fisiológica · diversidad funcional y taxonómica · capacidad metabólica

Características funcionais do bacterioplâncton cultivável de ambientes marinhos e estuarinos

Resumo. A informação sobre a estrutura do bacterioplâncton aumenta continuadamente enquanto que o conhecimento de sua capacidade metabólica continua limitado. Neste estudo se investigou a capacidade metabólica do bacterioplâncton, uma vez que tal informação é necessária para que se comprenda completamente o ciclo do carbono e outros processos biogeoquímicos. Foi estudada a diversidade das bactérias quimiorganotróficas cultiváveis que predominavam em um ambiente estuarino e em três marinhos isolando-se, aleatoriamente, unidades formadoras de colonias em meios sólidos e identificando-as taxonomicamente através da análise da seqüência de genes 16S rRNA e mediante a caracterização funcional dos isolados. A partir dos quatro locais amostrados foram obtidas 76 seqüências de genes 16S rRNA que representavam 19 genotipos diferentes. Os gêneros que foram isolados com maior freqüência foram: *Bacillus*, *Pseudomonas*, *Pseudoalteromonas*, *Vibrio* e *Erythrobacter*. A variação das funções metabólicas que tinha os conjuntos (*assemblages*) de bactérias cultivadas diferia notavelmente entre as distintas localidades amostradas. De maneira semelhante, em cada estação de amostragem, o percentual de bactérias que podia realizar alguma função específica era muito diferente para 18, das 25 funções metabólicas investigadas. Em dois locais, predominava um só gênero (*Pseudoalteromonas* ou *Erythrobacter*) os quais pareciam desempenhar funções especializadas. Mais de 95% dos isolados podiam utilizar aminoácidos e proteínas livres como única fonte de nitrogênio, e todos os isolados dos conjuntos especializados expressavam β-glucosidase. Somente alguns dos isolados podiam usar $NH4^+$, enquanto que uma terceira parte, de duas localidades marinhas, podiam crescer com NO_3^-. [**Int Microbiol** 2004; 7(3):219–227]

Palavras chave: bacterioplâncton cultivável · caracterização fisiológica · diversidade funcional e taxonômica · capacidade metabólica