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Biogeographical diversity among marine bacterioplankton

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ABSTRACT: The phylogenetic diversity of bacteria isolated on solid media from the Baltic Sea, Mediterranean Sea, Southern California Bight, Skagerrak, Weddell Sea (ice) and Andaman Sea was investigated by means of 16S rRNA gene sequence analysis. Of the 128 sequenced isolates, 52% showed similarity on the species level to previously reported bacteria, while as many as 18% showed a sequence similarity below 93%, which in the closest case would represent difference at the genus level. A majority of the isolated γ-Proteobacteria could be assigned to known species, while half of the α-Proteobacteria were only identified to the genus level. Bacteria affiliated with the Flexibacter-Cytophaga-Bacteroides phylum showed the lowest levels of similarity to previously sequenced bacteria, mainly representing novel genera. Closely related isolates most often originated from the same geographic area. Nevertheless, our data also demonstrated that most genera have closely related representatives widely distributed between different sea areas. Isolates related to environmental clones, with a sequence similarity above the tentative genus level, were found in 51 cases, of which 17 were more similar to clones than to cultured bacteria. From this result we concluded that a large proportion of the great species richness of marine bacteria, found by culture-independent techniques, is likely to be verified through information from live and functional bacteria.

KEY WORDS: Diversity · Bacterioplankton · 16S rRNA

INTRODUCTION

The paramount importance of microbial life for the cycling of matter in the oceans makes finding the identity and phylogenetic diversity of the indigenous bacteria an obvious research goal. Presently, the phylogenetic tree of marine bacterioplankton is rapidly growing. This is largely a result of extensive cloning of bacterial 16S rRNA genes from the environment, which has revealed a large number of novel taxonomic groups (Giovannoni et al. 1990, Fuhrman et al. 1992, 1993, DeLong et al. 1993, Mullins et al. 1995, Acinas et al. 1999). However, since a large majority of these novel taxa are not yet represented by bacteria in culture, the phenotypic expression of these bacteria remains unknown. Microbiologists came to the conclusion that a majority of the bacteria in the sea were unculturable since only a small proportion of the bacteria counted by epifluorescence microscopy were able to form colonies on solid media (Jannasch & Jones 1959, Kogure et al. 1979). The remaining bacteria were believed to be unable to grow in culture using then-present techniques. This bias against cultured bacteria has been an essential element in the decision to study cloned bacterial DNA instead of isolates of marine bacteria (Giovannoni et al. 1990, Fuhrman et al. 1992, Amann et al. 1995, Pace 1996, Fuhrman & Campbell 1998). However, another valid explanation for the discrepancy between total counts of bacteria and colony-forming units might be low plating efficiency due to virus infection or nutrient stress (Rehnstam et al. 1993, Nyström 1998). In line with this reasoning several recent studies using various hybridization protocols have demonstrated that bacteria that are able to grow on solid media occupy a significantly higher fraction of the bacterial community than the number of colonies

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on the plates would suggest (Rehnstam et al. 1993, Fuhrman et al. 1994, Moran et al. 1995, Faude & Höfle 1997, González & Moran 1997, Pinhassi et al. 1997, 1999, Tuomi et al. 1997, Weinbauer & Höfle 1998). Thus, significant species information from live and functional bacteria is currently being added to the phylogeny of marine bacteria.

Concurrent with the accumulation of data on the identity of marine bacteria, several authors have addressed questions regarding the biogeographic distribution of marine bacteria. Lee & Fuhrman (1991) made gross comparisons of the bacterial species composition using a whole community DNA hybridization technique, showing that 3 samples from widely separated sea areas (Pacific Ocean, Long Island Sound, Caribbean Sea) had less than 16% similarity. However, soon thereafter DeLong (1992) found similar Archaea in both Pacific and Atlantic US coastal waters, and Fuhrman et al. (1993) also reported 2 pairs of cloned sequences identical between the Southern California Bight and the Sargasso Sea (DeLong 1992, Fuhrman et al. 1993). Furthermore, Mullins et al. (1995) found several near-identical bacterial sequences from different oceans (Mullins et al. 1995). Based on this information these authors emphasized the presence of a few key microbial groups with widespread occurrence.

Throughout the brief period of marine bacterial phylogeny a major concern has been how to place the new clones and isolates into appropriate taxa. The current species concept in systematic bacteriology is based on the level of crosshybridization between genomic DNA of different strains of bacteria: if crosshybridization is >70%, the bacteria belong to the same species (Wayne et al. 1987). This species criterion was further defined by Stackebrandt & Goebel (1994), who demonstrated that bacteria with crosshybridization levels <70% never have a 16S rDNA sequence similarity >97%, and similar results have been presented by others (Devereux et al. 1990, Amann et al. 1992). In accordance with these data the intraspecies variability in the 16S rDNA for a number of bacteria have been reported to be low (<2%), although not necessarily zero (Wiik et al. 1995, Field et al. 1997, Yoon et al. 1998). Using these criteria as a basic assumption we previously demonstrated seasonal and short-term succession of different marine bacterial species (Pinhassi et al. 1997, 1999).

In this paper we present information on bacterial isolates collected from different sea areas, some of which have been shown to be important indigenous members of the marine bacterioplankton based on their demonstrated occurrence in the community DNA. In a simple manner the obtained species collection offered a possibility to present a first sketch of the phylogenetic affiliation and biogeographic distribution of cultured marine bacteria.

MATERIALS AND METHODS

Sampling and isolation of bacteria. Bacterial isolates were obtained by spreading samples of seawater onto Zobell agar plates. An effort was made to get representatives of all present colony morphologies in each sample. Samples were collected off the Thailand coast in the Andaman Sea (08°00' N, 98°00' E), from the Weddell Sea in Antarctica (65°00' S, 47°00' E), in the Skagerrak (58°56' N, 11°45' E), at point B in the bay of Villefranche-sur-Mer in the Mediterranean Sea (43°41' N, 7°19' E), off Scripps Pier in the Southern California Bight (32°53' N, 117°15' W), and at Stn NB1 in the northern Baltic Sea (63°30' N, 19°48' E). Pure cultures of the isolates were stored in glycerol (20% final concentration) at −70°C for subsequent phylogenetic characterization.

PCR-amplification, purification of PCR product and sequencing. 16S rRNA gene sequences of bacterial isolates from different sea areas were amplified by means of PCR (polymerase chain reaction) using *Taq* polymerase (Boehringer-Mannheim) from DNA preparations of cultured isolates. Bacterial 16S rDNA primers, 27f:biotinylated (AGAGTTTGATCATGGCTCAG) and 1492r (TACGGYTACCTTGTTACGACTT), were used for amplification (Giovannoni 1991). The reaction volumes were 50 µl, containing 1 µg template (genomic DNA), 10 mM total dNTP, standard 10× *Taq* buffer, a total of 15 ng of each primer, and 1 U of *Taq* polymerase. The PCR amplification conditions were as follows: 1 round of denaturation at 95°C (2 min), annealing at 50°C (30 s) and elongation at 72°C (45 s) and then 29 cycles of 95°C (30 s), 50°C (30 s) and 72°C (45 s) using a DNA thermal cycler 480 (Perkin Elmer). The biotinylated strand was purified using streptavidine-coated magnetic beads (Dynabeads M280-Streptavidine, Dynal AS, Norway). 25 µl of Dynabeads were washed once in TES-buffer (TE + 0.1 M NaCl), resuspended in 50 µl TES and incubated for 30 min with the 50 µl PCR reaction at room temperature. The beads were kept in suspension by gently tapping the tubes every 2 min. The biotinylated strand was separated by denaturing for 5 min with 100 µl 0.15 M NaOH and washing once with TES and once with water. The Dynabeads with the purified biotinylated strand were resuspended in 11 µl water. 16S rDNA nucleotide sequences were determined from the purified single stranded PCR product by automated sequencing, using ABI PRISM Dye Terminator Cycle Sequencing (Perkin Elmer) with primer 518r (CGTATTACCGCGGCTGCT) (Lane et al. 1985). The accession numbers for the 16S rRNA gene sequences of the isolates are presented in Table 1.

Phylogenetic analyses. The phylogenetic trees were constructed by a maximum parsimony method using

Table 1. Identity of bacterial isolates collected in different sea areas. Also indicated is the % sequence similarity of the isolates to previously reported organisms. Isolates marked in **bold** have previously been found to be dominant members of marine bacterioplankton. •Bacteria with sequence similarity values to 16S rRNA genes as close as (within 1.0%) or closer to environmental clones than to reported isolates. Similarity values are based on approximately 400 basepairs. In the taxon column, α, β, γ: different subclasses of the *Proteobacteria*; G+: Gram-positive bacteria; FCB: bacteria in the Flexibacter-Cytophaga-Bacteroides phylum

Isolate	Accession no.	Closest matching organism	Similarity (%)	Taxon
ANT1	AF025571	*Shewanella frigidimarina*; U85902	100.0	γ
ANT3•	AF025573	*Pseudomonas azotoformans*; D84009	99.4	γ
		Clone JAP501; U09827	99.3	γ
ANT6	AF025576	*Pseudoalteromonas* sp.; U85862	99.2	γ
		Hydrothermal vent clone PVB5; U15114	94.4	γ
ANT7	AF025577	*Moraxella* sp.; X86614	98.8	γ
ANT8	AF025578	*Pseudoalteromonas tetraodonis*; X82139	99.4	γ
		Hydrothermal vent clone PVB5; U15114	93.1	γ
SKA1	AF025314	*Colwellia* sp.; AF056462	99.8	γ
		Clone agg53; L10948	94.7	γ
SKA2	AF025315	*Cytophaga lytica*; M62796	96.4	FCB
SKA3	AF025316	*Roseobacter gallaeciensis*; Y13244	97.7	α
SKA4	AF025317	*Flavobacterium uliginosum*; M62799	93.9	FCB
SKA5	AF025318	*Flavobacterium uliginosum*; M62799	95.7	FCB
SKA6	AF025319	*Hyphomonas johnsonii*; AF082791	92.9	α
SKA7	AF025320	Marine psychrophile; U85886	92.5	FCB
SKA8	AF025321	*Hyphomonas johnsonii*; AF082791	92.4	α
SKA9	AF025322	*Pseudoalteromonas citrea*; AF082563	99.2	γ
		Hydrothermal vent clone PVB5; U15114	94.8	γ
SKA10	AF025323	*Roseobacter litoralis*; X78312	97.2	α
		Clone SMK735; X78652	94.2	α
SKA11	AF025324	*Marinomonas vaga*; X67025	91.3	γ
SKA12	AF025325	*Colwellia demingiae*; U85844	94.6	γ
		Clone agg53; L10948	93.4	γ
SKA13	AF025326	*Alpha proteobacterium*; AB015520	87.6	α
SKA14•	AF025327	*Stenotrophomonas maltophila*; X95924	98.8	β
		Clone MT3; AF058381	99.3	β
SKA16	AF025329	*Vibrio splendidus*; X74724	96.7	γ
SKA17	AF182014	*Arthrobacter histidinolovorans*; X83406	96.0	G+
MED1	AF025544	*Pseudoalteromonas* sp.; AF055816	98.5	γ
		Hydrothermal vent clone PVB5; U15114	97.0	γ
MED2	AF025545	*Shewanella woodyi*; AF003549	94.9	γ
MED3	AF025546	*Alteromonas* sp.; AB015135	98.7	γ
MED4•	AF025547	*Pseudoalteromonas* sp.; U85855	95.5	γ
		Hydrothermal vent clone PVB5; U15114	97.0	γ
MED5	AF025548	*Vibrio campbellii*; X74692	98.5	γ
MED6	AF025549	*Roseobacter algicola*; X78313	96.5	α
		ANG clone 1g; AF022395	94.9	α
MED9	AF025552	*Flavobacterium aquatile*; M62797	89.2	FCB
MED10	AF025553	*Psychroserpens burtonensis*; U62913	89.7	FCB
MED11	AF025554	*Psychroserpens burtonensis*; U62912	87.5	FCB
MED12	AF025555	*Psychrobacter glacincola*; U85877	99.3	γ
MED13	AF025556	*Agrobacterium sanguineum*; AB021493	97.6	α
		Clone SMK270; X78645	93.7	α
MED14	AF025557	*Pseudoalteromonas* sp.; U85862	98.6	γ
		Hydrothermal vent clone PVB5; U15114	94.2	γ
MED15•	AF025558	*Alteromonas macleodii*; X82145	93.7	γ
		Hydrothermal vent clone PVB12; U15115	93.1	γ
MED16	AF025559	*Brevundimonas subvibrioides*; AJ227784	98.5	α
MED17	AF025560	*Erythrobacter longus*; M59062	97.1	α
MED18	AF025561	*Polaribacter irgensii*; M61002	94.8	FCB
MED19	AF025562	*Shewanella pealeana*; AF011335	97.6	γ
MED20	AF025563	*Haererehalobacter ostenderis*; U78786	99.0	γ
MED21	AF025564	*Flexibacter* sp.; AB008044	96.5	FCB
MED22	AF025565	*Marinocaulobacter* sp.; AJ227808	97.6	α
MED23	AF025566	*Alteromonas macleodii*; Y18230	99.4	γ
		Clone UPB C7; AF111860	98.0	γ

(Table 1 continued on next page)

Table 1 (continued)

Isolate	Accession no.	Closest matching organism	Similarity (%)	Taxon
MED24*	AF025567	*Roseobacter litoralis*; X78312	94.5	α
		ANG clone 1a; AF022393	95.5	α
MED25	AF025568	Marine psychrophile; AF001368	91.1	FCB
MED26	AF025569	*Roseobacter litoralis*; X78312	96.5	α
		Clone SMK735; X78652	95.3	α
MED27	AF025570	*Pseudoalteromonas aurantia*; X82135	99.6	γ
		Hydrothermal vent clone PVB5; U15114	95.7	γ
MED28	AF182015	*Flexibacter* sp.; AB008044	96.0	FCB
AND1*	AF025957	*Alteromonas macleodii*; X82145	98.6	γ
		Clone DCM-ATT-9; AF114509	98.2	γ
AND2	AF025958	*Halomonas meridiana*; M93356	98.3	γ
AND4	AF025960	*Vibrio campbellii*; X74692	97.8	γ
AND5	AF025961	*Marinobacter* sp.; AJ000647	99.0	γ
AND6	AF025962	*Cytophaga marinoflava*; M58770	95.7	FCB
SCB8	**Z31657**	***Vibrio splendidus*; X74724**	**98.0**	**γ**
SCB11	**Z31658**	***Aeromonas hydrophila*; X87271**	**90.7**	**γ**
SCB21	U63998	*Sphingomonas* sp.; AJ011014	98.6	α
		Clone ASB003; AB010598	95.6	α
SCB22*	U63999	*Marinobacter* sp.; AJ000647	96.0	γ
		Clone OM59; U70695	96.7	γ
SCB23	U64000	*Chromohalobacter marismortui*; X87219	93.2	γ
SCB24	U64001	*Pseudomonas stutzeri*; U25280	99.8	γ
		Clone TRS3; AJ005873	98.3	γ
SCB25	U64002	*Mesorhizobium amorphae*; AF041442	97.2	α
		Clone LRE10; AJ232884	95.6	α
SCB26	U64003	*Erythrobacter longus*; M59062	96.3	α
		Clone GKS69; AJ224989	94.3	α
SCB27*	U64004	*Stenotrophomonas maltophila*; AJ131114	99.0	β
		Clone MT3; AF058381	98.4	β
SCB28*	**U64005**	***Roseobacter* sp.; AF022392**	**97.0**	**α**
		Clone GAC-2 ; AF007250	100.0	α
SCB29	U64006	*Vibrio mediterranei*; X74710	97.9	γ
SCB31*	**U64008**	***Roseobacter gallaeciensis*; Y13244**	**99.5**	**α**
		Egg clone D39; AF022397	98.9	α
SCB32	**U64009**	***Sulfitobacter pontiacus*; Y13155**	**98.3**	**α**
SCB33	**U64010**	***Alteromonas* sp.; AB015135**	**99.8**	**γ**
		Clone 400m-ATT-5; AF114507	98.2	γ
SCB34	**U64011**	***Roseobacter algicola*; X78315**	**96.3**	**α**
		ANG clone 1a; AF022393	94.9	α
SCB35	U64012	*Pseudoalteromonas nigrifaciens*; X82146	97.7	γ
SCB36	**U64013**	***Psychroserpens burtonensis*; Y17132**	**90.5**	**FCB**
SCB37	**U64014**	***Polaribacter irgensii*; M61002**	**91.7**	**FCB**
SCB38	**U64015**	***Cytophaga columnaris*; AB016515**	**89.7**	**FCB**
SCB39	U64016	*Vibrio tubiashi*; X74725	97.1	γ
SCB40	U64017	Marine bacterium; AJ002569	98.0	FCB
SCB41*	U64018	*Flavobacterium uliginosum*; M62799	93.0	FCB
		Clone KC429; AB022483	97.4	FCB
SCB42	**U64019**	***Octadecabacter antarcticus*; U14583**	**97.0**	**α**
SCB43*	**U64020**	***Pseudoalteromonas carrageenovora*; X82136**	**99.6**	**γ**
		Clone 400m-ATT-16; AF114522	98.8	γ
SCB44	**U64021**	***Polaribacter irgensii*; M61002**	**96.2**	**FCB**
SCB45*	**U64022**	***Halomonas campusalis*; AF054286**	**90.3**	**FCB**
		Clone T39; Z93986	93.7	FCB
SCB46	U64023	*Flexibacter* sp.; AB008044	95.2	FCB
SCB47	U64024	*Vibrio splendidus*; X74724	98.3	γ
SCB48*	**U64025**	***Roseobacter gallaeciensis*; Y13244**	**94.9**	**α**
		Egg clone D39; AF022397	95.0	α
SCB49	U64026	*Polaribacter irgensii*; M61002	87.1	FCB
SCB50	U64027	*Marinomonas vaga*; X67025	96.5	γ
SCB51	AF026390	*Vibrio campbellii*; X74692	97.6	γ
SCB52*	AF026391	*Burkholderia solanacearum*; S55002	91.8	β
		Clone OM156; U70706	92.6	β

Table 1 (continued)

Isolate	Accession no.	Closest matching organism	Similarity (%)	Taxon
SCB53	AF026392	Beta proteobacterium; AF035052	93.4	β
SCB56	AF052601	*Alteromonas macleodii*; Y18234	98.9	γ
		Clone SUR-ATT-13; AF114497	98.0	γ
SCB57	AF182016	*Microbacterium* sp.; AB010618	95.0	G+
SCB58*	AF182017	*Kocuria rhizophila*; Y16264	95.7	G+
		CloneMT2; AF058372	97.8	G+
SCB59	AF182018	*Sulfitobacter pontiacus*; Y13155	99.8	α
BAL2*	U63934	*Rhodobacter azotoformans*; D70846	95.5	α
		Clone LRS3; AJ232852	96.6	α
BAL3	**U63935**	***Brevundimonas* sp.; AJ227801**	**99.5**	**α**
BAL4	U63936	*Flavobacterium* sp.; U85890	92.0	FCB
BAL5	U63937	*Sphingomonas* sp.; U37345	98.1	α
BAL6	AF182023	Unid. actinomycete; AB015562	97.1	G+
BAL7	AF182024	*Acinetobacter radioresistens*; X81666	98.6	γ
BAL9	U63938	*Flavobacterium* sp.; AB008041	99.5	FCB
		Clone LBS17; AJ232835	93.8	FCB
BAL10	AF182019	*Sphingomonas subarctica*; X94102	97.5	α
BAL11	**U63939**	***Rhizomonas suberifaciens*; D13737**	**97.7**	**α**
BAL13	U63940	*Flavobacterium* sp.; U85889	95.7	FCB
BAL15*	U63941	*Janthinobacterium lividum*; Y08846	99.4	β
		Clone CTHB-18; AF067655	99.2	β
BAL16*	U63942	*Pseudomonas megulae*; AF074383	97.1	γ
		Clone NB1-g; AB013828	97.3	γ
BAL17	**U63943**	***Cytophaga aquatile*; M58764**	**95.0**	**FCB**
BAL18*	**U63944**	***Pseudomonas veronii*; AF064460**	**99.8**	**γ**
		Clone TRS26; AJ005872	99.6	γ
BAL22*	**U63946**	***Flavobacterium aquatile*; M62797**	**91.8**	**FCB**
		Clone LBS17; AJ232835	92.2	FCB
BAL23	**U63947**	***Pseudomonas gessardi*; AF074384**	**99.8**	**γ**
BAL25	U63948	*Shewanella baltica*; AJ000216	97.9	γ
BAL27*	U63949	*Rhodobacter azotoformans*; D70847	95.4	α
		Clone LRS3; AJ232852	96.3	α
BAL29	**U63950**	**Marine psychrophile; U85891**	**92.2**	**FCB**
BAL31	**U63951**	***Pseudomonas anguilliseptica*; X99541**	**97.3**	**γ**
		Clone LRE23; AJ232872	96.1	γ
BAL34	U63952	*Agrobacterium sanguineum*; AB021493	98.7	α
		Clone TRS1; AJ006014	93.9	α
BAL37	**U63953**	***Alcaligenes* sp.; AJ002802**	**97.7**	**β**
BAL38*	U63954	*Flavobacterium aquatile*; M62797	92.0	FCB
		Clone LBS17; AJ232835	92.3	FCB
BAL39*	U63955	*Flavobacterium heparinum*; M11657	94.4	FCB
		Clone JAP411; U09780	96.4	FCB
BAL40*	U63956	*Sphingomonas terrae*; D13727	95.3	α
		Clone ASB003; AB010598	94.3	α
BAL43	U63957	*Zoogloea ramigera*; X74915	95.6	α
		Clone SJA-53; AJ009467	93.8	α
BAL44	**U63958**	***Sphingomonas capsulata*; D16147**	**93.6**	**α**
BAL45	U63959	*Sphingomonas* sp.; X94098	97.5	α
BAL46	U63960	*Sphingomonas* sp.; U52146	96.3	α
BAL47*	**U63961**	***Rhodoferax fermentans*; D16211**	**92.3**	**β**
		Clone 4-4; AJ011158	96.4	β
BAL48	U63962	*Sphingomonas* sp.; U52146	97.6	α
		Clone LRE17; AJ232863	95.6	α
BAL49	AF182020	Marine psychrophile; U85891	95.4	FCB
BAL50*	AF182021	*Cytophaga* sp.; X85210	93.5	FCB
		Clone LBS24; AJ232834	93.3	FCB
BAL51	AF182022	*Brevundimonas* sp.; AJ227801	100.0	α
BAL52	AF182025	*Hyphomonas* sp.; M83812	86.8	α
BAL53	AF182026	*Sphingomonas yanoikuyae*; D16145	92.1	α
BAL54	AF182027	*Sphingomonas yanoikuyae*; D16145	97.7	α
BAL55	AF182028	*Pseudomonas putida*; D37923	98.5	γ

PAUP 3.0 for the Macintosh. The trees were calculated from the nucleotide positions approximately corresponding to the positions 130 to 500, *Escherichia coli* numbering. Our isolate sequences were compared to existing procaryotic sequences in the GenBank using the 'fasta' command in the GCG-package. Closest matching clones were obtained by the similarity rank command in RDP (Ribosomal Database Project).

DNA-DNA relatedness. Cross hybridization between the different bacteria was assessed by hybridization of extracted DNA. The DNA from the different isolates was blotted onto hybridization membranes according to the manufacturer (Hybond-N, Amersham), using a slot blot apparatus (GIBCO BRL). The membranes were then hybridized towards single whole genome probes prepared by labelling the genomic DNA prepared from each of the different bacteria with a nick translation kit (Promega) and [α-^{32}P]dATP (Amersham) (Pinhassi et al. 1999, 1997). The hybridization signal was detected and quantified using a PhosphorImager (Molecular Dynamics).

RESULTS

Over a number of years we collected approximately 500 bacterial isolates from the Baltic Sea (BAL), Mediterranean (MED), Southern California Bight (SCB), Skagerrak (SKA), Weddell Sea ice (ANT) and the Andaman Sea (AND). Based on differences in colony morphology we selected 215 isolates to be sequenced, out of which 128 different isolates are included in the present analysis (Table 1). The remaining sequenced isolates were replicates or near identical and were omitted for the sake of clarity. The extensive list in Table 1 includes 26 species that have been demonstrated to be dominant components of the community DNA at different locations. For example SCB8 and SCB11 showed bloom occurrence in the waters off Scripps Pier (Rehnstam et al. 1993), SCB37 and SCB54 achieved high numbers in 2 seawater mesocosm experiments (Pinhassi et al. 1999), and BAL8 and BAL11 occurred at high abundance during different times of the year in the Baltic Sea (Pinhassi et al. 1997).

Ideally bacterial systematics should rely on both genotypic and phenotypic characteristics, i.e. a polyphasic approach (Wayne et al. 1987, Murray et al. 1990). For this study, however, we elected to use only 16S rRNA sequence information since this would allow a comparison to environmental clones that, due to the lack of phenotypic traits, have been assigned taxonomic affiliation solely on 16S rRNA sequence information. Partial sequences of the 16S rRNA gene (350 to 400 bases) were used in the analysis. Therefore a comparison of the variability of the full-length sequence and the partial sequence was made to ensure that the partial sequences were long enough to resolve taxonomic differentiation between isolates. Sequences from closely related isolates of each of the Cytophaga, α-Proteobacteria and γ-Proteobacteria groups were aligned and the corresponding similarity values were recorded. Furthermore, sequences were compared between groups, thus generating alignments with lower match. The resulting dataset from 16 comparisons was evaluated using a linear regression showing a high degree of correlation between full length and partial sequences (r^2 = 0.975, $p < 0.005$). On average the difference in similarity was less than 1%. A series of measurements on the crosshybridization between different isolates was carried out to establish a level of sequence similarity required to tell different species and genera apart. From the results we could compile the degree of DNA-DNA relatedness versus the similarity between the respective 16S rRNA gene sequences (Fig. 1), although a few outliers were present in the dataset. From this graph, and similar data from previous compilations (Devereux et al. 1990, Amann et al. 1992, Stackebrandt & Goebel 1994), we decided that isolates with a sequence similarity of ≥97% were representatives of the same species. Furthermore, a similarity between 97 and 93% would represent identity at the genus level but difference at the species level. The minimum level of sequence similarity chosen for our tentative genus designation (>93%) was a conservative limit based on the taxonomic discussion of genus identity among 3 different bacterial groups (Devereux et al. 1990, Dobson et al. 1993, Wiik et al. 1995).

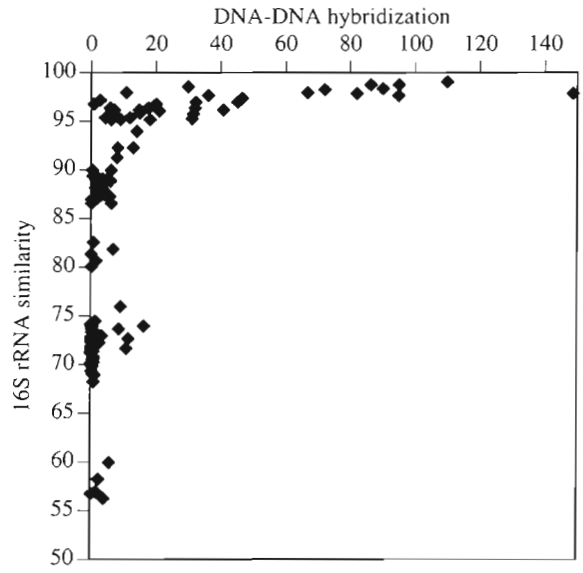

Fig. 1. Comparison of DNA-DNA hybridization between different isolates and the corresponding sequence similarity of partial 16S rRNA genes

Table 2. Sequence similarity of the isolates to closest matching relative in the data bank. Distribution of isolates from the different divisions among sequence similarity values representing new genus (<0.93), new species within previously characterized genus (0.93 to 0.97), and previously characterized species (>0.97)

Bacterial division	Sequence similarity to previously reported bacteria			No. of isolates
	>0.97	0.93–0.97	<0.93	
γ-Proteobacteria	78%	18%	4%	46
α-Proteobacteria	55%	33%	12%	40
Flexibacter-Cytophaga-Bacteroides	7%	43%	50%	30
β-Proteobacteria	57%	14%	29%	7
Gram-positive bacteria	25%	75%	0	4
All divisions	52%	30%	18%	126

The sequence similarity values of our isolates to previously reported organisms are presented in Table 1. Half of our isolates showed a 16S rRNA gene sequence similarity of <97% to previously reported sequences, and 18% showed similarities ranging from 82 to 93% (Table 2). These low similarity values indicate novelty at the genus level. The Flexibacter-Cytophaga-Bacteroides phylum appeared as the least known taxon, whereas the γ-Proteobacteria generally showed high similarity values to known bacteria.

Environmental clones related to our isolates were included in Table 1 when the sequence similarity suggested a relationship above the tentative genus level. This was the case for 51 isolates, of which 17 were more similar to clones than to cultured bacteria. For example, SCB28 was 100% similar to environmental clone GAC-2 while being 97% similar to a *Roseobacter* isolate.

The α-Proteobacteria. Fig. 2 shows a phylogenetic tree of the isolated α-Proteobacteria. A majority of these isolates were divided into 2 separate lineages, largely representing the genera *Roseobacter* and *Sphingomonas*. A prominent feature was the lack of *Roseobacter* isolates in the Baltic Sea, and the lack of bacteria with origin other than the Baltic Sea within the *Sphingomonas*. Although in a distant branch, the Southern California Bight isolate SCB21 and the Baltic isolate, BAL40, also produced high similarity to known *Sphingomonas*. Among the *Roseobacter*, isolates were found from the Southern California Bight, Mediterranean Sea, and Skagerrak. A separate cluster was also formed by 3 different *Hyphomonas* species from the Skagerrak. Near identical sequences of bacteria collected in different sea areas were found in 2 cases: the *Roseobacter* isolates from the Southern California Bight (SCB34) and the Mediterranean Sea (MED6) were close to identical and from the same sea areas, and the *Erythrobacter* isolates SCB26 and MED17 were 99.1% similar.

The γ-Proteobacteria. Almost half of our isolates belonged to the γ-Proteobacteria (Fig. 3). Two-thirds of these isolates clustered into 5 major groups representing the genera *Vibrio*, *Pseudoalteromonas*, *Alteromonas*, *Shewanella*, and *Pseudomonas*. In the genera *Pseudoalteromonas* and *Alteromonas*, closely related bacteria from several different sea areas were found. Within the genera *Vibrio*, *Shewanella*, and *Pseudomonas*, isolates from different sea areas were found, but the heterogeneity within the genera was higher and no identical isolates originating from different sea areas were found. A striking feature was the lack of isolates from the Baltic Sea among the genera *Vibrio*, *Pseudoalteromonas*, and *Alteromonas*. The Baltic isolates were instead found among *Pseudomonas*, *Shewanella*, and the *Acinetobacter*. The genus *Acinetobacter* was found together with a number of halotolerant and psychrophilic bacterial genera in a deeply rooted cluster, possibly related at the family level.

The Flexibacter-Cytophaga-Bacteroides phylum. The bacterial isolates affiliated with this phylum were distributed among 4 major lineages (Fig. 4). The bacteria from the Baltic Sea were mainly divided into 2 major clusters separated from bacteria of other geographic origins. One lineage represented bacteria close to *Flexibacter* and the other represented a subcluster of the Cytophaga-Flavobacterium branch. Several of the Southern California Bight isolates clustered with Mediterranean isolates, although only 1 instance of close relationship between the Southern California Bight and the Mediterranean was found in this group: SCB46 and MED21 showing a sequence similarity of 99.4%. Three other Southern California Bight isolates (SCB36, -37, -49) formed a loose cluster with *Polaribacter* sp. as the closest neighbour. The isolates from the Mediterranean clustered separately. Specific lineages separated at the genus level were also characteristic of 7 of the 8 isolates from the Southern California Bight.

The Gram-positive and β-Proteobacteria. Only a few Gram-positive isolates were found in the different sea areas (Table 2, Fig. 5). The isolates belonging to the β-Proteobacteria (Fig. 5) were also few, but may represent biogeochemically interesting species, since several of them are reported to have the ability to perform nitrogen fixation (Malik & Schlegel 1981).

DISCUSSION

In this study the diversity of bacteria isolated on solid media from a number of different sea areas was investigated by means of 16S rRNA gene sequencing. The

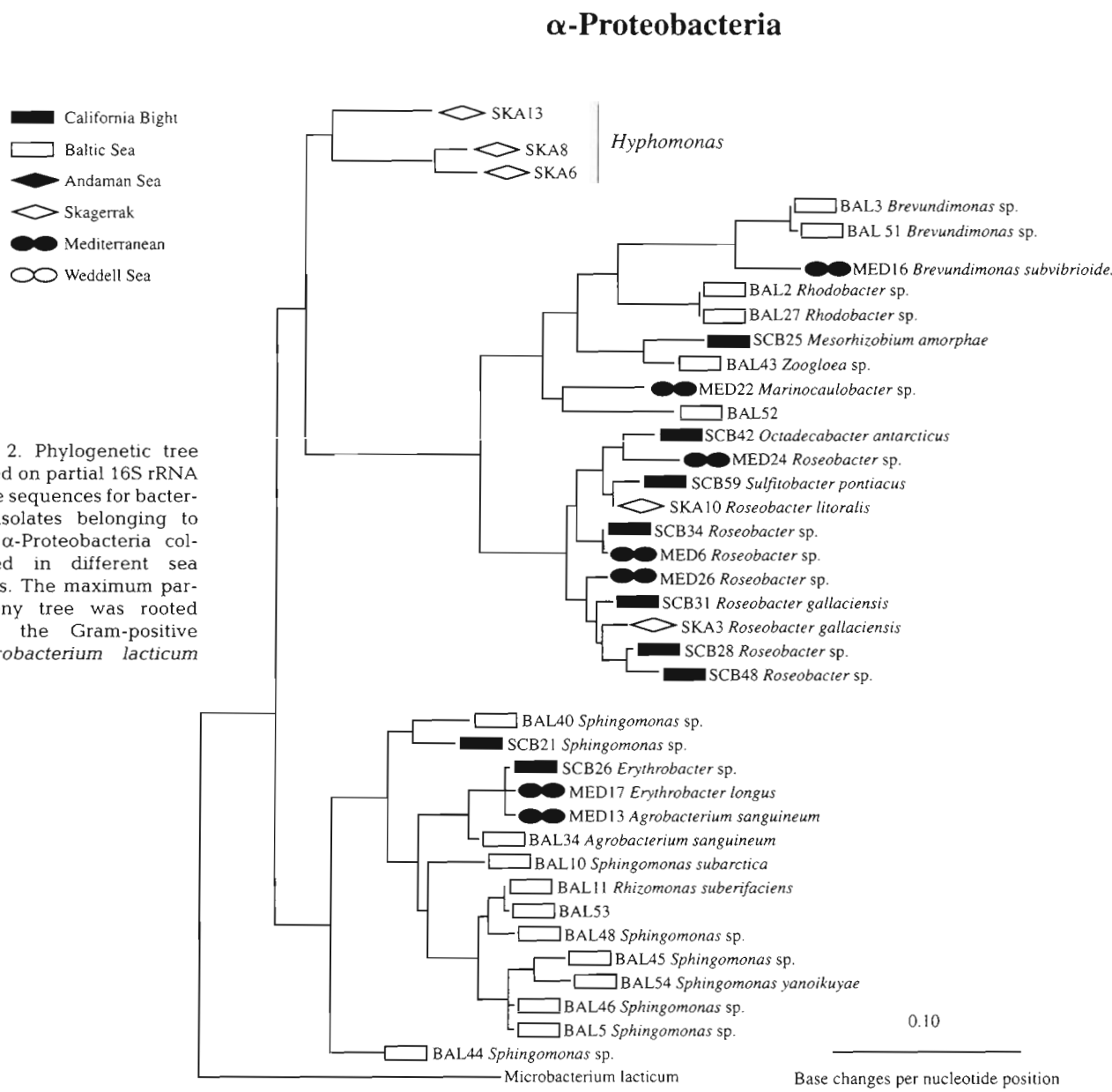

Fig. 2. Phylogenetic tree based on partial 16S rRNA gene sequences for bacterial isolates belonging to the α-Proteobacteria collected in different sea areas. The maximum parsimony tree was rooted with the Gram-positive *Microbacterium lacticum*

phylogenetic analyses revealed a considerable species diversity among the isolated bacteria, with sequence similarity values of the isolates to previously reported sequences ranging from 82 to 100% (Table 1). Notably, half of the isolates showed a sequence similarity of <97% to previously reported sequences, with a high proportion (18%) having sequence similarities ranging from 82 to 93% (Table 2). These low similarity values indicate novelty at the genus level, and even at the level of new families (Devereux et al. 1990). The incidence of poorly characterized taxa was highest for members of the Flexibacter-Cytophaga-Bacteroides phylum, whereas the γ-Proteobacteria generally showed high similarity values to known bacteria. These observations are in agreement with other recent reports on the prevalence of novel bacteria among bacteria in culture (Rehnstam et al. 1993, Bowman et al. 1997, Kalmbach et al. 1997, Pinhassi et al. 1997, Suzuki et al. 1997). In a collection of isolates from Pacific waters off the coast of Oregon, a majority of the isolates showed sequence similarities, ranging from 84 to 97%, to previously reported nearest neighbours in the RDP database (Suzuki et al. 1997). Similarly, in a study of the phylogenetic diversity among bacterial isolates from Antarctic sea ice, 26 phylogenetic groups putatively equivalent to bacterial genera were found, including several apparently novel genera (Bowman et al. 1997). Thus, the great bacterial diversity in the marine envi-

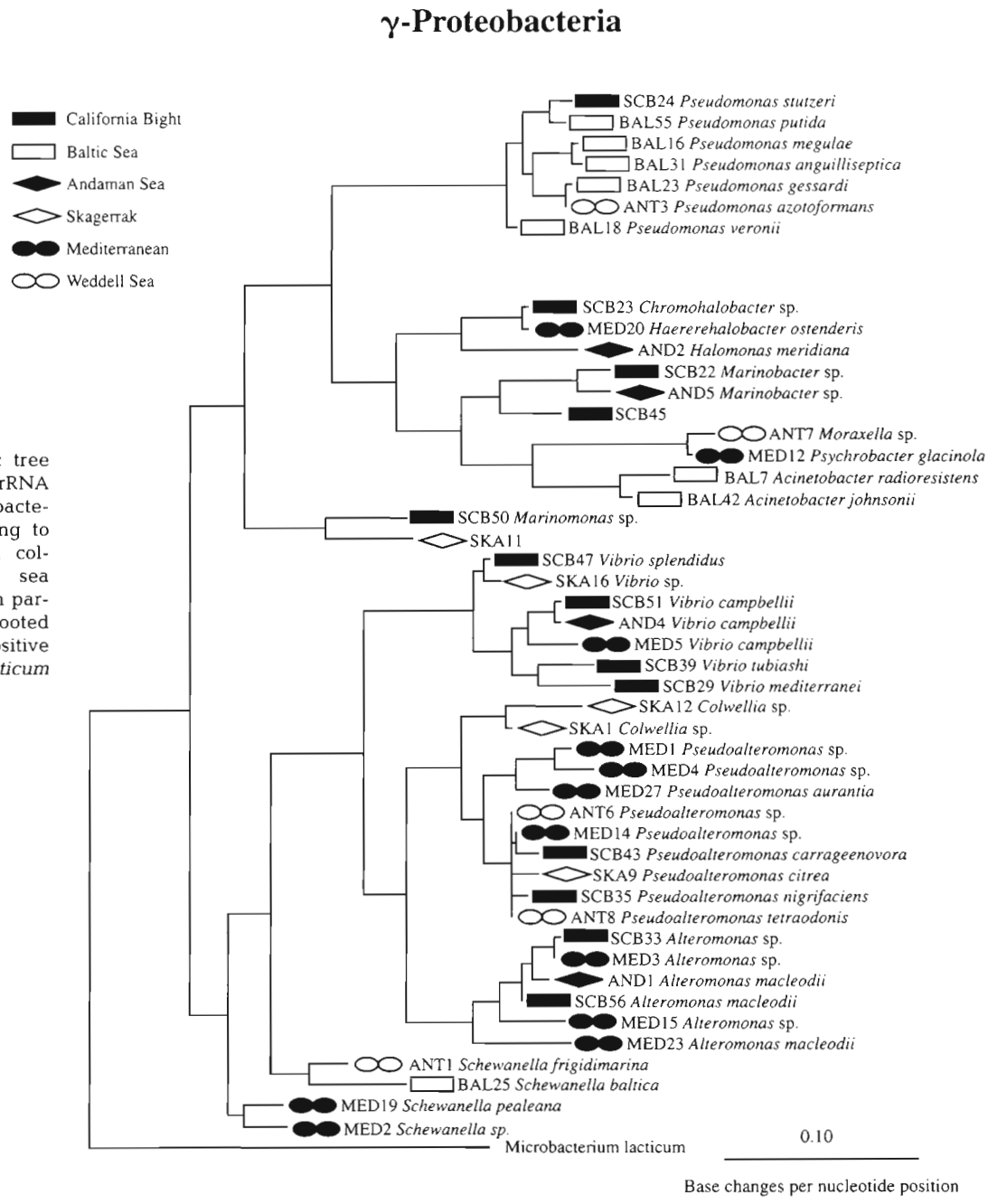

Fig. 3. Phylogenetic tree based on partial 16S rRNA gene sequences for bacterial isolates belonging to the γ-Proteobacteria collected in different sea areas. The maximum parsimony tree was rooted with the Gram-positive *Microbacterium lacticum*

ronment found by culture-independent techniques can be extended using information from live and functional bacteria.

One-third of the bacteria in this study belonged to the γ-Proteobacteria, and a significant proportion of the isolates belonged to the α-Proteobacteria and the Flexibacter-Cytophaga-Bacterioides phylum. The predominance of γ-Proteobacteria in the sequence databases as well as in other work on bacteria in culture (Mullins et al. 1995, Bowman et al. 1997, Suzuki et al. 1997) is in contrast to their scarcity when assessed by culture-independent methods (Giovannoni et al. 1990, DeLong et al. 1993, Fuhrman et al. 1993). In our experience γ-Proteobacteria rapidly form conspicuous colonies, while several of the α-Proteobacteria grow slowly and produce small, nondescript colonies easy to overlook on agar plates. This could be one explanation for the abundance of γ-Proteobacteria in culture collections. The poor match to reported sequences of our bacteria matching *Cytophaga* relatives was, however, unexpected, given the interest in the enzymatic versatility of this taxon, as well as their often vivid coloration.

A number of the isolated bacteria have previously been shown to occupy significant fractions of the bac-

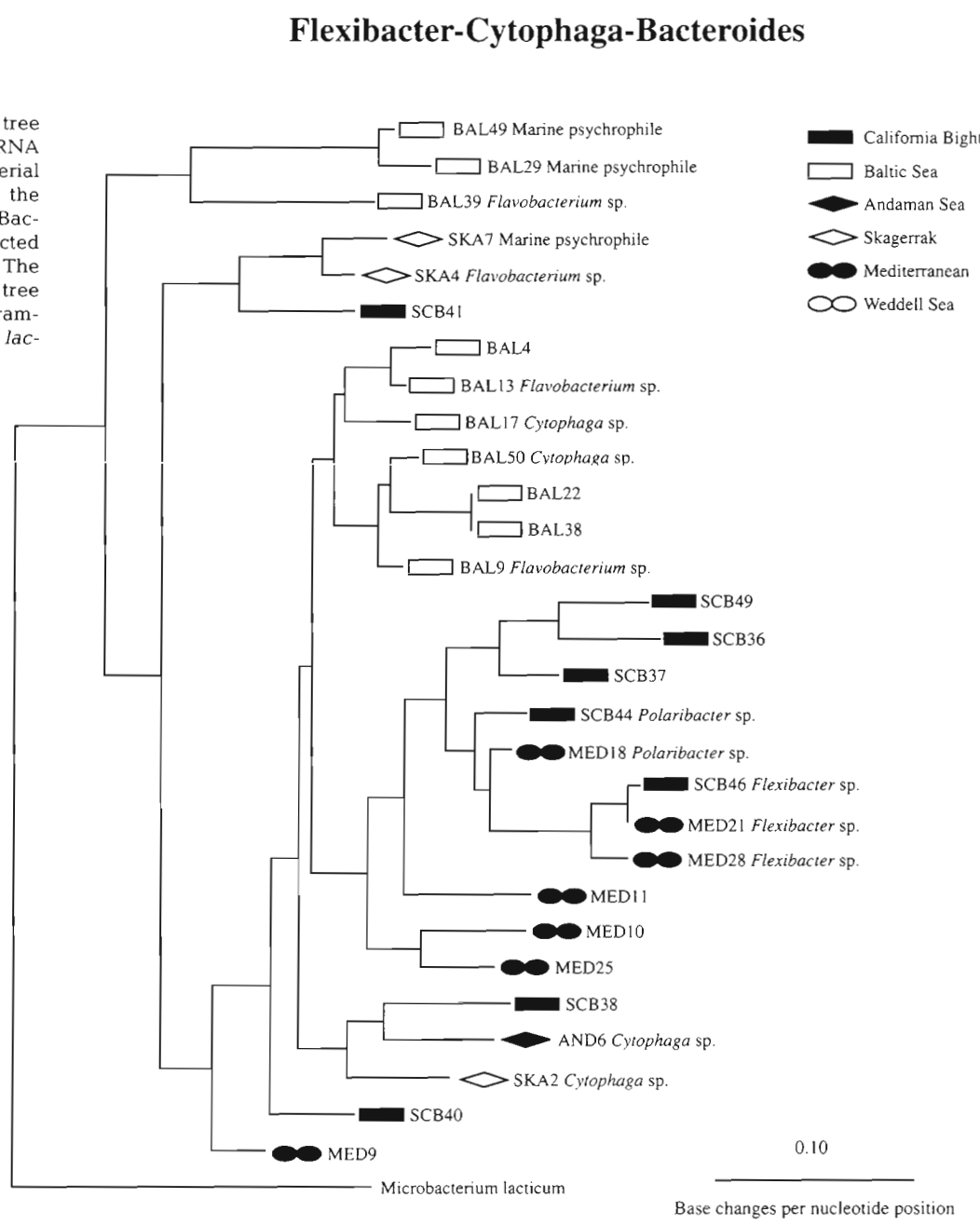

Fig. 4. Phylogenetic tree based on partial 16S rRNA gene sequences for bacterial isolates belonging to the Flexibacter-Cytophaga-Bacteroides phylum collected in different sea areas. The maximum parsimony tree was rooted with the Gram-positive *Microbacterium lacticum*

terioplankton in different environments (Rehnstam et al. 1993, Pinhassi et al. 1997, 1999, Pinhassi & Hagström 2000, in this issue) (see Table 1). It was therefore interesting to investigate the possible degree of similarity of the isolates to organisms detected through environmental cloning. This search showed that 13% of the isolates showed higher sequence similarities to cloned sequences than to cultured bacteria. This value is in line with the data presented by Suzuki et al. (1997) comparing the sequences of 26 different isolated bacteria to 25 sequences obtained by cloning from the same seawater sample. Their comparison revealed that 3 of the sequences were shared by isolates and clones (Suzuki et al. 1997). Similar results were found by Chandler et al. (1997), who showed that the bacterial isolates accounted for 11% of the environmental clones, although the number of different clones was 5 times the number of isolates (Chandler et al. 1997). Agreement between cultivation and direct cloning techniques have also been demonstrated for the methanogenic community in soil (Großkopf et al. 1998). Thus, it appears likely that increased efforts to cultivate bacteria from the environment will yield increasing insights into the indigenous bacterial diversity.

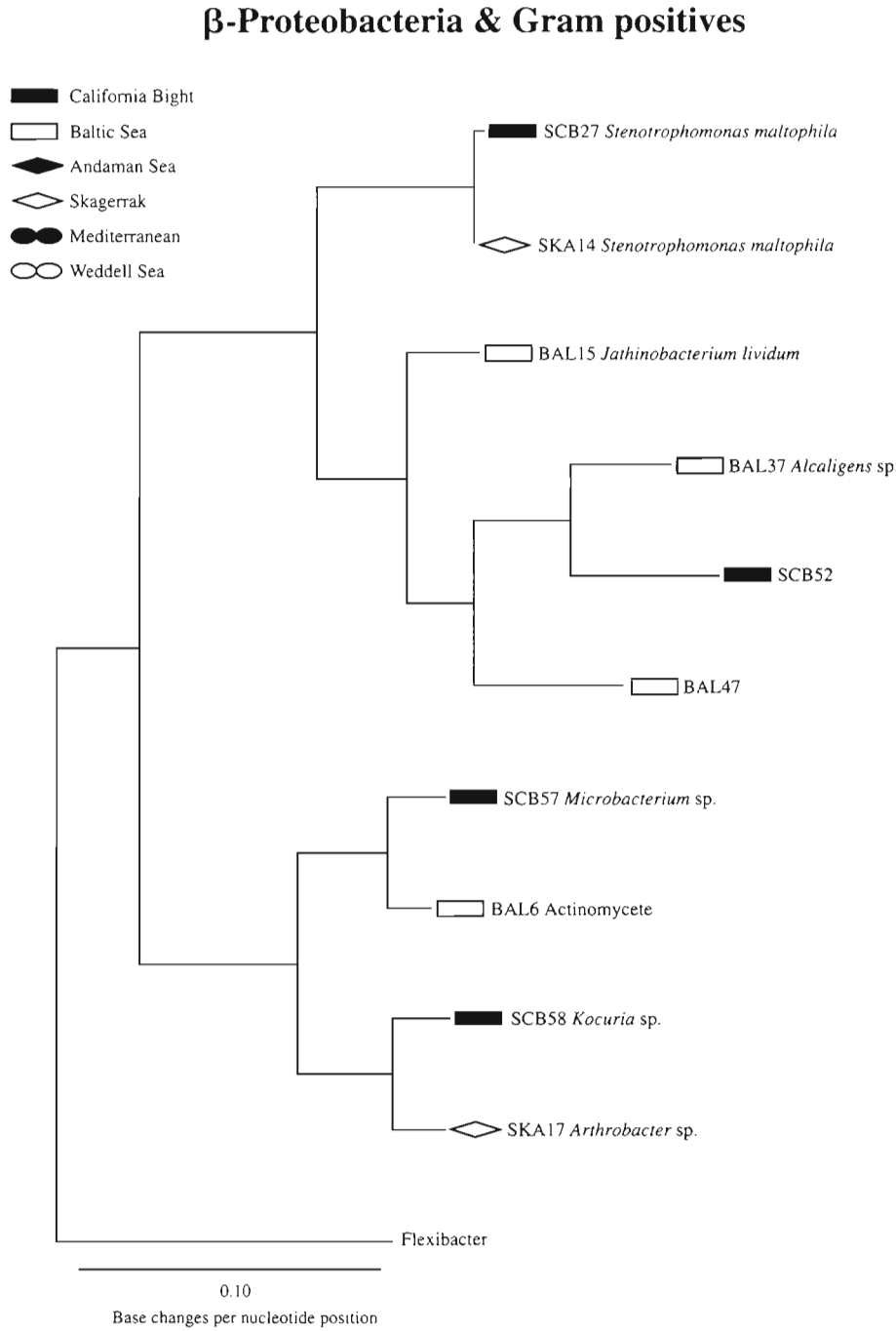

Fig. 5. Phylogenetic tree based on partial 16S rRNA gene sequences for bacterial isolates belonging the β-Proteobacteria and to a few Gram-positive bacteria collected in different sea areas. The maximum parsimony tree was rooted with a Gram-negative *Flexibacter* species

Geographic distribution of the isolates

Bacterioplankton community composition in different sea areas was studied by Lee & Furhman (1991). Using a whole community DNA hybridization technique, they found a low similarity between Pacific Ocean, Long Island Sound and Caribbean Sea samples (<16%). This result suggested that few bacterioplankton species would be distributed worldwide. However near identical environmental clones from different sea areas have subsequently been reported by DeLong (1992), Fuhrman et al. (1993), and Mullins et al. (1995), although they in no case represented the majority of the studied clones. Also, Bowman et al. (1997) found nearly identical isolates in Antarctic sea ice and temperate environments. In the present study, closely related isolates most often originated from the same geographic area. Most genera could be found in the Southern California Bight, the Skagerrak and the northwest Mediterranean, although represented by

different species. This is illustrated by the isolates belonging to the genus *Pseudoalteromonas*, which showed a high degree of similarity between different sea areas, and the *Vibrio* species that seem more specific to each sea area. In the phylogenetic trees the Baltic Sea isolates often formed separate lineages, representing different genera. It is likely that the lack of 'marine' (salt tolerant) genera can be attributed to the low salinity in the northern Baltic (<10‰). Species of *Roseobacter* and *Vibrio* as well as *Alteromonas* all have a salt requirement for growth. In their place members of the genera *Pseudomonas, Shewanella,* and *Sphingomonas* are common in the Baltic. The presence of the first 2 genera has been demonstrated previously (Höfle & Brettar 1995, 1996). In the Baltic the salinity gradient is a very stable property and the different community composition (at the genus level) is therefore not unexpected. Instead it is tempting to suggest that other estuarine environments may differ from oceanic environments in the same manner. The Weddell Sea ice represents a seemingly isolated habitat, and may not be expected to harbour bacteria that would occur in other sea areas. However, the Baltic isolate BAL23 belonging to the genus *Pseudomonas* was almost identical to the Weddell Sea ice isolate ANT3, and together with a second close couple related to *Shewanella* served to link these 2 remote sea areas. Also, within the genus *Pseudoalteromonas*, 2 isolates, 1 from the Southern California Bight and 1 from the Mediterranean Sea, were found to be closely related to a Weddell Sea ice isolate.

Divergence and persistence of bacterial species

Widespread species distribution between different sea areas raises the question of how this 'homogeneity' among certain genera is maintained. In order to answer this question several complex issues, such as spreading of surface seawater, mechanisms for transfer of genetic material and the resulting population genetics, have to be considered. Although significant changes in the bacterioplankton species composition occur at various temporal and spatial scales, the persistence of specific bacteria between consecutive years seems to be a general phenomenon (Lee & Fuhrman 1991, Rehnstam et al. 1993, Höfle & Brettar 1995, Faude & Höfle 1997, Ziemke et al. 1997, Murray et al. 1998). It has also been demonstrated that stratification can result in more pronounced differences between different depths sampled in 1 yr than the inter-annual difference at the same depth (Murray et al. 1998). This suggests genetically stable and persistent bacterioplankton. However, hard evidence of genetic stability in bacterioplankton populations over long time periods will be difficult to provide, but examples from other sources can be found. An interesting case of genetic stability is seen in the *rpoB* and *pla* gene sequences of the *Yersinia pestis* (the cause of plague). Samples from victims of the epidemics 400 yr ago have been obtained and compared to present isolates, and the comparison demonstrates complete identity of the 2 genes (Drancourt et al. 1998).

It has been argued that the gradual divergence that will inevitably occur as the result of minor mutational events and reading errors will require recombination as a stabilizing mechanism (Cohan 1995). If true, this genetic exchange has the potential to constrain intracluster divergence. Transduction is believed to be an important mechanism for transfer of DNA in bacterioplankton, and thereby potentially recombination (Bergh et al. 1989, Fuhrman & Suttle 1993, Jiang & Paul 1998). Jiang & Paul (1998) estimated the frequency of transduction to be between 0 and 100 transductants l^{-1} d^{-1} in an area off the coast of Florida. Although seemingly small, these numbers are significant when considering extended time periods and large areas. These estimates were obtained by a transduction model using a single phage-host system (Jiang & Paul 1998). In a concurrent study of a large number of phage-host systems isolated in the North Sea, Wichels et al. (1998) demonstrated that multiple phage species can infect the same *Pseudoalteromonas* species. This would suggest even higher transduction frequencies in complex communities, giving support to the notion of frequent recombination.

When entire genes are transferred the prevailing selection pressure may result in the acquisition of new genetic traits. However, it may be argued that the bacterial chromosome can resist neutral change when no selection pressure is acting. This can be seen in the high degree of conservation in genome size and gene order. Selective constraints operate on the bacterial chromosome in order to maintain a basic organization. Examples of such constraints are the need for the terminus to oppose the origin of replication to minimize chromosomal replication rates (Riley & Sanderson 1990), and the maintenance of an advantageous orientation of transcription relative to the direction of replication (Brewer 1990). Further constraints have been outlined by Bergthorsson & Ochman (1997). Thus, to compensate for any negative effects that could be caused by changed genome structure the added benefit of the information provided by the new DNA must be considerable. Extrachromosomal DNA, on the other hand, can be allowed to reside in the cell with less constraints and mediate exchangeable potential features to the cell.

Bacterioplankton require physical transport of water parcels for efficient genetic transfer to occur over large

sea areas. Eilola (1998) produced a valuable estimate of horizontal transfer of water, using spreading of juvenile fresh water as a tracer. When comparing these results obtained in the Baltic Sea to tracer experiments in other sea areas, Eilola arrived at an average spreading velocity of 5 cm s^{-1}. This means that within months surface waters will spread and mix over large sea areas (Eilola 1998). A second important route for the transfer of bacterial DNA is sea spray (Blanchard & Syzdek 1970, Baylor et al. 1977). Bacteria, as well as potentially transducing phages, could be transported at windspeeds typically ranging from 5 to 20 m s^{-1}, which means a 100-fold higher transfer rate than surface water transport.

In the present study, we concluded that although a few species could be found widely distributed in different oceans, more commonly 'worldwide' distributions could be assigned to bacteria at the genus level. This result is in agreement with a low degree of community DNA hybridization between samples from different sea areas (Lee & Fuhrman 1991), and the records of ubiquitous identical clones (DeLong 1992, Fuhrman et al. 1993, Mullins et al. 1995). An encouraging observation in our study was the high proportion of cultured isolates that demonstrated high similarity to reported environmental clones. This indicates a wealth of diverse indigenous bacteria yet to be explored under culture conditions.

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LITERATURE CITED

Acinas SG, Antón J, Rodríguez-Valera F (1999) Diversity of free-living and attached bacteria in offshore western Mediterranean waters as depicted by analysis of genes encoding 16S rRNA. Appl Environ Microbiol 65:514–522

Amann RI, Lin C, Key R, Montgomery L, Stahl DA (1992) Diversity among *Fibrobacter* isolates: towards a phylogenetic classification. Syst Appl Microbiol 15:23–31

Amann RI, Ludwig W, Schleifer KH (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev 59:143–169

Baylor ER, Baylor MB, Blanchard DC, Syzdek LD, Appel C (1977) Virus transfer from surf to wind. Science 198: 575–580

Bergh Ø, Börsheim KY, Bratbak G, Heldal M (1989) High abundance of viruses found in aquatic environments. Nature 340:467–468

Bergthorsson U, Ochman H (1997) Evolution of the *E. coli* genome. In: de Bruijn FJ, Lupski JR,Weinstock GM (eds) Bacterial genomes: physical structure and analysis. Chapman & Hall, New York, p 177–186

Blanchard DC, Syzdek L (1970) Mechanism for the water-to-air transfer and concentration of bacteria. Science 170: 626–628

Bowman JP, McCammon SA, Brown MV, Nichols DS, McMeekin TA (1997) Diversity and association of psychrophilic bacteria in Antarctic sea ice. Appl Environ Microbiol 93:3068–3078

Brewer (1990). Replication and the transcriptional organisation of the *Escherichia coli* chromosome. In: Drlica K, Riley M (eds) The bacterial genome. American Society for Microbiology, Washington, DC, p 61–83

Chandler DP, Li SM, Spadoni M, Drake GR, Balkwill DL, Fredrickson JK, Brockman FJ (1997) A molecular comparison of culturable aerobic heterotrophic bacteria and 16S rDNA clones derived from a deep subsurface sediment. FEMS Microbiol Ecol 23:131–144

Cohan FM (1995) Does recombination constrain neutral divergence among bacterial taxa? Evolution 49:164–175

DeLong EF (1992) Archaea in coastal marine environments. Proc Natl Acad Sci USA 89:5685–5689

DeLong EF, Franks DG, Alldredge AL (1993) Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. Limnol Oceanogr 38:924–934

Devereux R, He SH, Doyle CL, Orkland S, Stahl DA, LeGall J, Whitman WB (1990) Diversity and origin of *Desulfovibrio* species: phylogenetic definition of a family. J Bacteriol 172:3609–3619

Dobson SJ, McMeekin TA, Franzmann PD (1993) Phylogenetic relationships between some members of the genera *Deleya*, *Halomonas* and *Halovibrio*. Int J Syst Bacteriol 43: 665–673

Drancourt M, Aboudharam G, Signoli M, Dutour O, Raoult D (1998) Detection of 400-year-old *Yersinia pestis* DNA in human dental pulp: an approach to the diagnosis of ancient septicemia. Proc Natl Acad Sci USA 95: 12637–12640

Eilola K (1998) Oceanographic studies of the dynamics of fresh water, oxygen and nutrients in the Baltic Sea. Thesis, Publ A30 1998, Gothenburg University

Faude UC, Höfle MG (1997) Development and application of monoclonal antibodies for in situ detection of indigenous bacterial strains in aquatic environments. Appl Environ Microbiol 63:4534–4542

Field KG, Gordon D, Wright T, Rappé M, Urbach E, Vergin K, Giovannoni SJ (1997) Diversity and depth-specific distribution of SAR11 cluster rRNA genes from marine planktonic bacteria. Appl Environ Microbiol 63:63–70

Fuhrman JA, Campbell L (1998) Microbial microdiversity. Nature 393:410–411

Fuhrman JA, Suttle CA (1993) Viruses in marine planktonic systems. Oceanography 6:51–63

Fuhrman JA, McCallum K, Davis AA (1992) Novel major archaebacterial group from marine plankton. Nature 356: 148–149

Fuhrman JA, McCallum K, Davis AA (1993) Phylogenetic diversity of subsurface marine microbial communities from the Atlantic and Pacific oceans. Appl Environ Microbiol 59:1294–1302

Fuhrman JA, Lee SH, Masuchi Y, Davis AA, Wilcox RM (1994) Characterization of marine procaryotic communities via DNA and RNA. Microb Ecol 28:133–145

Giovannoni SJ (1991). The polymerase chain reaction. In: Stackebrandt E, Goodfellow M (eds) Sequencing and hybridization techniques in bacterial systematics. John

Wiley & Sons, Inc, New York, p 177–201
Giovannoni SJ, Britschgi TB, Moyer CL, Field KJ (1990) Genetic diversity in Sargasso Sea bacterioplankton. Nature 345:60–63
González JM, Moran MA (1997) Numerical dominance of a group of marine bacteria in the alpha-subclass of the class *Proteobacteria* in coastal seawater. Appl Environ Microbiol 63:4237–4242
Großkopf R, Janssen PH, Liesack W (1998) Diversity and structure of the methanogenic community in anoxic rice paddy soil microcosms as examined by cultivation and direct 16S rRNA gene sequence retrieval. Appl Environ Microbiol 64:960–969
Höfle M, Brettar I (1995) Taxonomic diversity and metabolic activity of microbial communities in the water column of the central Baltic Sea. Limnol Oceanogr 40:868–874
Höfle MG, Brettar I (1996) Genotyping of heterotrophic bacteria from the central Baltic Sea by use of low-molecular-weight RNA profiles. Appl Environ Microbiol 62: 1383–1390
Jannasch HW, Jones GE (1959) Bacterial populations in sea water as determined by different methods of enumeration. Limnol Oceanogr 4:128–139
Jiang SC, Paul JH (1998) Gene transfer by transduction in the marine environment. Appl Environ Microbiol 64: 2780–2787
Kalmbach S, Manz W, Szewzyk U (1997) Isolation of new bacterial species from drinking water biofilms and proof of their in situ dominance with highly specific 16S rRNA probes. Appl Environ Microbiol 63:4164–4170
Kogure K, Simidu U, Taga N (1979) A tentative direct microscopic method for counting living marine bacteria. Can J Microbiol 25:415–420
Lane DL, Pace B, Olsen GJ, Stahl DA, Sorgin ML, Pace NR (1985) Rapid determination of 16S rRNA sequences for phylogenetic analysis. Proc Natl Acad Sci USA 82: 6955–6959
Lee S, Fuhrman JA (1991) Spatial and temporal variation of natural bacterioplankton assemblages studied by total genomic DNA cross-hybridization. Limnol Oceanogr 36: 1277–1287
Malik KA, Schlegel HG (1981) Chemolithoautotrophic growth of bacteria able to grow under N_2-fixing conditions. FEMS Microbiol Lett 11:63–67
Moran MA, Rutherford LT, Hodson RE (1995) Evidence for indigenous *Streptomyces* populations in a marine environment determined with a 16S rRNA probe. Appl Environ Microbiol 61:3695–3700
Mullins TD, Britschgi TB, Krest RL, Giovannoni SJ (1995) Genetic comparisons reveal the same unknown bacterial lineages in Atlantic and Pacific bacterioplankton communities. Limnol Oceanogr 40:148–158
Murray AE, Preston CM, Massana R, Taylor LT, Blakis A, Wu K, DeLong EF (1998) Seasonal and spatial variability of bacterial and archaeal assemblages in the coastal waters near Anvers Island, Antarctica. Appl Environ Microbiol 64:2585–2595
Murray RGE, Brenner DJ, Colwell RR, De Vos P, Goodfellow M, Grimont PAD, Pfenning N, Stackebrandt E, Zavarzin GA (1990) Report of the ad hoc committee on approaches to taxonomy within the protobacteria. Int J Syst Bacteriol 40:213–215
Nyström T (1998) To be or not to be: the ultimate decision of the growth-arrested bacterial cell. FEMS Microbiol Rev 21:283–290
Pace NR (1996) New perspectives on the natural microbial world: molecular microbial ecology. ASM News 62: 463–470
Pinhassi J, Hagström Å (2000) Seasonal succession in marine bacterioplankton. Aquat Microb Ecol 21:245–256
Pinhassi J, Zweifel UL, Hagström Å (1997) Dominant marine bacterioplankton species found among colony-forming bacteria. Appl Environ Microbiol 63:3359–3366
Pinhassi J, Azam F, Hemphälä J, Long RA, Martinez J, Zweifel UL, Hagström Å (1999) Coupling between bacterioplankton species composition, population dynamics, and organic matter degradation. Aquat Microb Ecol 17: 13–26
Rehnstam AS, Bäckman S, Smith DC, Azam F, Hagström Å (1993) Blooms of sequence-specific culturable bacteria in the sea. FEMS Microbiol Ecol 102:161–166
Riley M, Sanderson KE (1990) Comparative genetics of *Escherichia coli* and *Salmonella typhimurium*. In: Drlica K, Riley M (eds) The bacterial genome. American Society for Microbiology, Washington, DC, p 85–95
Stackebrandt E, Goebel BM (1994) Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Syst Bacteriol 44:846–849
Suzuki MT, Rappé MS, Haimberger ZW, Winfield H, Adair N, Ströbel J, Giovannoni SJ (1997) Bacterial diversity among small-subunit rRNA gene clones and cellular isolates from the same seawater sample. Appl Environ Microbiol 63: 983–989
Tuomi P, Torsvik T, Heldal M, Bratbak G (1997) Bacterial population dynamics in a meromictic lake. Appl Environ Microbiol 63:2181–2188
Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murray RGE, Stackebrandt E, Starr MP, Trüper HG (1987) Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. Int J Syst Bacteriol 37:463–464
Weinbauer MG, Höfle MG (1998) Distribution and life strategies of two bacterial populations in a eutrophic lake. Appl Environ Microbiol 64:3776–3783
Wichels A, Biel SS, Gelderblom HR, Brinkhoff T, Muyzer G, Schütt C (1998) Bacteriophage diversity in the North Sea. Appl Environ Microbiol 64:4128–4133
Wiik R, Stackebrandt E, Valle O, Daae FL, Rødseth OM, Andersen K (1995) Classification of fish-pathogenic Vibrios based on comparative 16S rRNA analysis. Int J Syst Bacteriol 45:421–428
Yoon JH, Lee ST, Park YH (1998) Inter- and intraspecific phylogenetic analysis of the genus *Nocardioides* and related taxa based on 16S rDNA sequences. Int J Syst Bacteriol 48: 187–194
Ziemke F, Brettar I, Höfle MG (1997) Stability and diversity of the genetic structure of a *Shewanella putrefaciens* population in the water column of the central Baltic. Aquat Microb Ecol 13:63–74

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