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Microbial Community Analysis of Aquatic Gliding Bacteria (*Cytophaga-Flavobacterium-Bacteroides*) by Fluorescence In Situ Hybridization (FISH)

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Abstracts

16S rRNA-targeted oligonucleotide probes for the specific detection of the phylum *Cytophaga-Flavobacterium-Bacteroides* (CFB) were designed and used to monitor the distribution of gliding bacteria in marine specimens from Songkhla lake. The probes were checked for their specificity and then validated using fluorescent in situ hybridization (FISH) with target microorganisms and non-target microorganisms. *Tenacibaculum mesphilum*, *Rapidithrix thailandica*, *Aureispira marina*, *Aureispira maritima*, *Fulvivirga kasyanovii*, and *Cytophaga* sp. were detected with the probe, and hybridization percentage was less than 50 % for all 6 probes tested. Plant material contained between 8.3×10^{9} to 7.4×10^{10} cells ml^{-1}, seagrass contained between 6.4×10^{10} to 6.8×10^{10} cells ml^{-1}, biofilms contained between 2.1×10^{9} to 4.6×10^{11} cells ml^{-1}, dead fish contained between 1.9×10^{10} to 2.6×10^{10} cells ml^{-1}, seaweed contained between 1.3×10^{10} to 1.8×10^{10} cells ml^{-1} and sponge contained 2.9×10^{10} cells ml^{-1}. Members of the *Cytophaga-Flavobacterium* group were found in some marine specimens, and biofilm samples, except for SK9 and SK11, and usually formed the largest bacterial group in the marine specimens. The medians of the relative abundances and the absolute cell numbers of the alpha-subclass *Proteobacteria* detected with the probe ALF1b were significantly higher in the marine samples, except for SK7, SK9, and SK11. The results showed the good applicability of the probes TENA, RAP, AUNA, AUMA, FUL, and CYTO for the specific detection and quantification of *T. mesphilum, R. thailandica, A. marina, A. maritima, F. kasyanovii*, and *Cytophaga* sp. in complex environments.

Keywords: FISH, marine gliding bacteria, designed probes, *cytophaga-flavobacterium-bacteroides*, marine specimens

Introduction

Several attempts have already been made to describe marine sediment microbial communities. Most of these have been based on cultivation [1] and were, therefore, subject to restriction sand biases, leading to a distorted representation of the true community composition [2]. Molecular techniques have greatly increased knowledge of marine microbial diversity. During the last few years, numerous efforts have been made to increase the sensitivity of fluorescent in situ hybridization (FISH), including the use of brighter fluorochromes [3,4], image-intensified video microscopy, chloramphenicol treatment to increase the rRNA content of the growing bacterial fraction [5,6], hybridization with more than one fluorescently labeled oligonucleotide probe [7], helper oligonucleotide probes, multiply labeled polyribonucleotide probes, and signal amplification with reporter enzymes [8].

Gliding bacteria are Gram-negative, non-phototrophic, and non-flagellated. They exhibit a creeping or gliding motility on solid surfaces. The organisms are not filamentous; instead, the vegetative cells are

unicellular short rods, resembling typical bacteria, except the walls are flexible. Gliding, nonfruiting bacteria may appear as individual rods or filaments (e.g., *Cytophaga*, *Flexibacter*, or *Vitreoscilla*), or as multicellular trichomes (e.g., *Beggiatoa*, *Simonsiella*, *Saprospira*, or *Thiothrix*). Many species can degrade natural polymers such as cellulose, chitin, pectin, keratin, or even agar, and are excellent producers of complex natural products, a fact which cannot be related to their taxonomy due to the phylogenetic heterogeneity described so far [9].

Kearns [10] reported that gliding bacteria are new microbial sources of natural products that are urgently needed in order to find new structural classes and/or unique structural variations. Gliding bacteria, as found in the taxa *Chloroflexi*, *Proteobacteria*, *Bacteroidetes*, and *Cyanobacteria*, include members whose secondary metabolism have hardly been investigated. Another important group of gliding bacteria, generally designated as the *Cytophaga-Flavobacterium* branch, is found in the phylum *Bacteroidetes*. For a long time, the differentiation of genera within the *Bacteroidetes* relied on the presence or absence of gliding motility.

The aim of this study was to estimate the bacterial composition and diversity of Songkhla Lake, which contains brackish water of about half the salinity of the ocean, *via* partial sequencing and taxon-specific oligonucleotide probing of 16S rRNA and development of higher order probes for the specific characterization of bacteria belonging to the CFB phylum.

Materials and methods

Sample collection and preparation

Marine specimen samples were collected from Songkhla Lake (7^o 45' N to 7^o 55' N 100^o 05' E to 100^o 15' E) located in the vicinity of Songkhla city, Thailand, during October 2011 (**Table 1**). Samples were collected from 6 sampling intertidal areas along Songkhla Lake. This water surface is actually a lagoon complex geologically. The marine specimens were briefly rinsed in sterile seawater of the same salinity as the sampling area and used within 48 h after collection.

The bacteria were then detached from the surfaces with a sterile razor blade and dispersed aseptically by sonication for 2 min. The homogenized marine samples (1 g) were suspended in 9 ml of filtered PBS. This suspension was diluted 10 times in filtered PBS and thoroughly mixed. The supernatant (375 µl) was collected and fixed overnight at 4 °C with 1125 µl of 4 % (w/v) filtered paraformaldehyde solution. The suspension was centrifuged at 10,000 rpm for 5 min at 4 °C. The fixed cells were then washed twice with filtered 0.1 mol/l PBS, at pH 7. Finally, the pellets were suspended in 150 µl PBS, mixed with 150 µl ice-cold ethanol, and stored at –20 °C until further analysis. The prepared sample could be kept for up to 3 months.

Design of 16S rRNA targeted oligonucleotide probes

Six oligonucleotide probes were designed specific to the *T. mesphilum* (TENA), *R. thailandica* (RAP), *A. marina* (AUNA), *A. maritima* (AUMA), *F. kasyanovii* (FUL), and *Cytophaga sp.* (CYTO). Oligonucleotide probes were designed by using the Primer 3Plus program. The specifics of the probes were reverified by comparison with the databases of National Center for Biotechnology Information (NCBI). The database ProbeBase was used for information on oligonucleotide probes known to date [11]. Criteria for the sequence of oligonucleotide probes were 18 - 20 bases and no self-complementary structures[12]. The secondary structure of 16S rRNA was also taken into consideration to confirm the potential target within the single stranded region of the native ribosome. Designed probes were tested using the probematch function of the Ribosomal Database Project (RDP) software package version 9 [13]. The probes were named according to the nomenclature proposed by the Oligonucleotide Probe Database (OPD) [14,15]. All probes were synthesized commercially (Thermo Hybaid, Germany) and were 5' labelled with the Cy3 sulfoindo-cyanine fluorochrome. EUB338 served as a positive control in permeability tests [16].

Table 1 List of marine specimen samples.

Code	Sample
SK1	unidentified plant material
SK2	unidentified plant material
SK3	seagrass
SK4	unidentified-plant material
SK5	sponge
SK6	biofilm on other substrate
SK7	dead fish
SK8	seaweed
SK9	biofilm on plastic
SK10	biofilm on other substrate
SK11	biofilm on other substrate
SK12	biofilm on plastic
SK13	biofilm on other substrate
SK14	unidentified plant material
SK15	seaweed
SK16	seagrass
SK17	biofilm on other substrate
SK18	dead fish

Detection and quantification the bacteria by FISH

The newly designed probes were used to quantify 6 gliding species in the marine specimen samples. In addition, the microbial composition of the gliding bacteria was determined by FISH, with 3 domain specific oligonucleotide probes (EUB338 targeting *Bacteria*, ALF1b targeting *Alpha-Proteobacteria*, and CF319a targeting *Cytophaga-Flavobacterium*).

Cell fixation

Cells were harvested by centrifugation (2 min, 5000×*g*) and carefully suspended in 1 ml sterile phosphate-buffered saline at pH 7.4 (PBS). The supernatant was collected (375 µl) and fixed overnight at 4 °C with 1125 µl of 4 % (w/v) filtered paraformaldehyde solution. Cells were stored in a 1:1 mixture of PBS and 96 % (v/v) ethanol at –20 °C. Three volumes of 4 % paraformaldehyde fixative were added to one volume of sample and held at 4 °C for 12 h [17,18]. Cells were pelleted by centrifugation (5000×*g*) and the fixative removed. Cells were washed in 1 % PBS and resuspended in 1 % PBS to give 10^8 - 10^9 cells ml^{-1}. One volume of ice-cold 100 % ethanol was added and mixed. The fixed samples were stored at –20 °C.

In situ hybridization

Each sample (20 µl) was dropped on to a poly-l-lysine slide and dried at room temperature. Slides were dehydrated for 3 min in 50, 80, and 100 % (v/v) ethanol, respectively. After, the dehydration slides were covered with 8 µl of prewarmed (46 °C) hybridization solution containing 0.9 M NaCl, 20 mM Tris-HCl (pH 7.2), 5 mM ethylenediamine tetraacetic acid (EDTA), 0.01 % (w/v) sodium dodecyl sulfate (SDS), a variable concentration of formamide, and 100 ng of each oligonucleotide probe. Hybridization buffer was placed in a 2 ml microcentrifuge tube [3].

Domain and group specific probes were used, as shown in **Table 2**. Eight µl of hybridization buffer were added to of a poly-l-lysine slide containing a sample and 0.5 µl of each probe (50 ng/µl). Slides were placed in the 50 ml tube containing paper soaked in hybridization buffer. The tubes were sealed and placed horizontally into a hybridization oven at 46 °C for 3 h. Unbound probes were removed by 10 ml of washing buffer (700 µl of 5 M NaCl, 1 ml of 1 M Tris-HCl, autoclaved milli-Q water up to 50 ml, and 50 µl of 10 % SDS), and incubated at 48 °C for 15 min, followed by MilliQ washing 3 times.

Slides were dried on paper and stained with DAPI solution for 5 min at room temperature in the dark. Subsequently, they were gently washed in sterile water, dried, and the mounted slides viewed using oil immersion. FISH was conducted with Cy3-labeled oligonucleotide probes. Samples were hybridized with the probes EUB338, ALF1b, CF319a, TANA, RAP, AUNA, AUMA, FUL, and CYTO, as described previously [3,22].

Table 2 Oligonucleotide probes used in this study.

Probe	Specificity	Sequence (59 - 39) of probe	Target site (rRNA positions)	% FA in situ	References
EUB338	*Bacteria*	GCTGCCTCCCGTAGGAGT	16S (338 - 355)	0 - 35	2
ALF968	Alpha subclass of *Proteobacteria*	GGTAAGGTTCTGCGCGTT	16S (968 - 986)	35	40
CF319a	*Cytophaga-Flavobacterium* cluster of CFB phylum	TGGTCCGTGTCTCAGTAC	16S (319 - 336)	35	31
TENA	*Tenacibaculum mesphilum*	ATCCCCCACACTGGTACTGA	16S, 328 - 347	-	Probe design
RAP	*Rapidithrix thailandica*	TATACGGGAAGAACTCCA-C	16S, 491 - 510	-	Probe design
AUNA	*Aureispira marina*	GAAGGCAGTGCACTAGGGCA	16S, 775 - 794	-	Probe design
AUMA	*Aureispira maritima*	GTTGTGCGTCCCATTAGCTA	16S, 525 - 544	-	Probe design
FUL	*Fulvivirga kasyanovii*	CTAACTCCGTGCCAGCAGCC	16S, 553 - 572	-	Probe design
CYTO	*Cytophaga sp.*	TAAGGGCTACGTGTAG-CTTG	16S, 504 - 523	-	Probe design

Microscopy and documentation

Mounted slides were prepared using an anti-fading solution, Citifluor. Bacterial cells on the slide were observed with an E-400 epifluorescent microscope (Nikon Eclipse 80i, USA) equipped with optimal filter sets (UV-2A for DAPI, HQ:CY3 for Cy3). For each probe and sample, between 700 and 1,000 DAPI-stained cells and the respective hybridized cell (10 to 20 independent fields) were counted [19,21]. Probe specific cell counts were presented as the percentage of cells visualized by DAPI, and the mean abundance and standard deviations were calculated.

Results and discussion

Probe design

Specificity of the designed probes was tested with 6 pure strains of gliding bacteria cultivated cells, which showed that all probes displayed the anticipated specificity (data not shown). Successful probe penetration through grown cells was evaluated with both EUB338 and the specific probes for each target strain. The sequences and details of the probes used in this study are presented in **Table 2**. On the results shown, hybridization percentage was less than 50 % for all probes design tested. The low percentage of hybridization may vary depending on the target organisms, specific to each probe and percentage of paraformaldehide in fixation. Key elements of the procedure are: adequate fixation, optimal permeabilization, and careful selection of the best combination between the probe, a fluorescence label, and formamide concentration [20]. Valm *et al*. [21] reported that the region from positions 319 to 336 of

the 16S rRNA was ascertained to be conserved for most of the bacteria belonging to the *Cytophaga-Flavobacterium* group. About 80 % of the species in this group have a C residue at position 328 of their 16s rRNA sequences; the remainder have a U residue at the corresponding position.

The design of new group specific probes for members of CFB-phylum turned out to be difficult. A good group-specific probe, which was complementary to most of the members of the CFB-phylum and to none or only a few outgroup organisms, could not be found. CF319a is, at least for the *Cytophaga-Flavobacterium* branch, still the most general probe available. However, [22] reported that, the larger the number of available sequences is, the more deviations from those idiosyncrasies are found. This problem also applies to already published group-specific probes, like those for the alpha, beta, and gamma subclasses of the class *Proteobacteria.* Our study was intended to supplement the set of group-specific probes with respect to the CFB-phylum. It attempted to design new probes that should at least target most of the members of the CFB-phylum that had been isolated from specific habitats. Ultimately, we hope that the set of probes designed will be suggestive for a quite narrow, potentially coherent target group, very much like other molecular fingerprints.

Bacterial community structure

There were abundances of major bacterial groups. For a set of 9 probes for major phyla within the domain *Bacteria*, all probe-specific cell counts were presented as the percentage of cells visualized by DAPI. The total cell counts found in the samples (10^9 to 10^{11} cells ml^{-1}) were in the normal range reported for marine and aquatic systems, as shown in **Table 3**. The detection sensitivity of FISH with EUB338 probe was higher than DAPI staining. All samples examined showed bright hybridization signals and a clear distinction between probe-conferred signals and the background (**Figures 1** and **2**).

Table 3 Total cell counts and relation of hybridized cells with specific probes.

Samples		Probes and Total cell count (Log Cells/ml)			
		EUB	**DAPI**	**ALF1b**	**CF319a**
SK1	unidentified plant material	9.66 ± 1.10	9.96 ± 1.34	5.91 ± 2.08	7.58 ± 0.83
SK2	unidentified plant material	10.06 ± 1.60	10.86 ± 1.82	5.92 ± 1.87	7.86 ± 0.95
SK3	seagrass	10.05 ± 2.26	10.80 ± 2.19	6.42 ± 1.27	7.46 ± 1.14
SK4	unidentified plant material	9.46 ± 0.94	10.07 ± 2.5	6.17 ± 2.28	7.29 ± 1
SK5	sponge	10.05 ± 2.19	10.46 ± 0.70	7.78 ± 2.22	7.81 ± 2.12
SK6	biofilm on other substrate	10.18 ± 2	10.18 ± 1.41	6.34 ± 2.75	8.73 ± 1.91
SK7	dead fish	9.25 ± 1.64	10.26 ± 1.66	-	8.69 ± 2.06
SK8	seaweed	9.25 ± 1.14	10.11 ± 1.81	7.73 ± 1.51	8.55 ± 1.59
SK9	biofilm on plastic	9.22 ± 1.15	9.32 ± 1.03	-	-
SK10	biofilm on other substrate	9.23 ± 1.64	10.07 ± 0.70	6.37 ± 1.78	7.99 ± 2.82
SK11	biofilm on other substrate	9.12 ± 1.73	9.49 ± 1.12	-	-
SK12	biofilm on plastic	9.17 ± 1.47	10.22 ± 0.89	5.95 ± 1.76	8.85 ± 1.30
SK13	biofilm on other substrate	10.76 ± 1.41	11.66 ± 2.06	6.41 ± 2.85	7.26 ± 2.16
SK14	unidentified plant material	9.84 ± 1.32	9.92 ± 2.75	6.55 ± 2.36	7.30 ± 1.89
SK15	seaweed	10.11 ± 1.93	10.25 ± 2.23	7.86 ± 1.59	8.76 ± 1.31
SK16	seagrass	10.06 ± 1.52	10.83 ± 1.52	7.98 ± 1.82	7.14 ± 1
SK17	biofilm on other substrate	9.68 ± 2.36	10.07 ± 3.36	6.53 ± 2.16	7.32 ± 1.25
SK18	dead fish	10.10 ± 1.11	10.41 ± 1.80	6.50 ± 1.75	8.65 ± 1.30

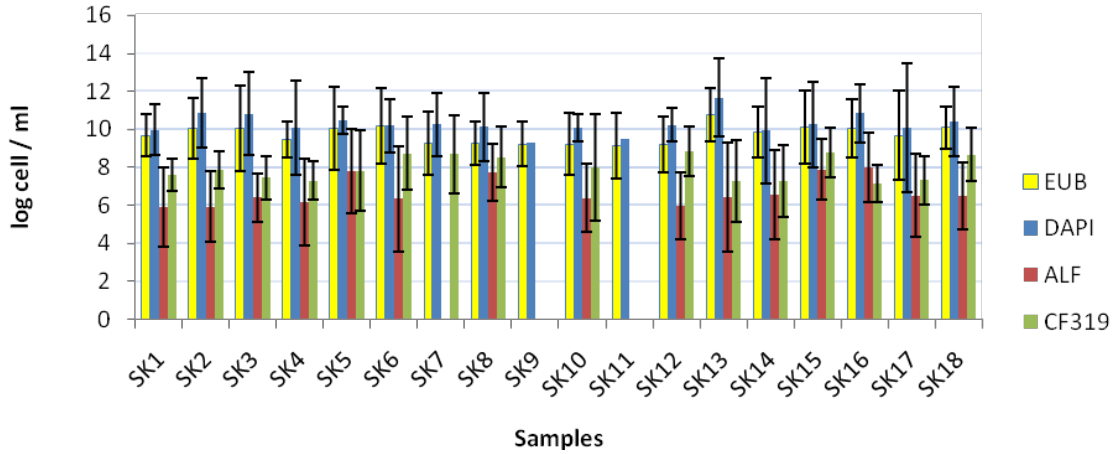

Figure 5 Cell counts of FISH-stained bacteria of 4 different probes per milliliter.

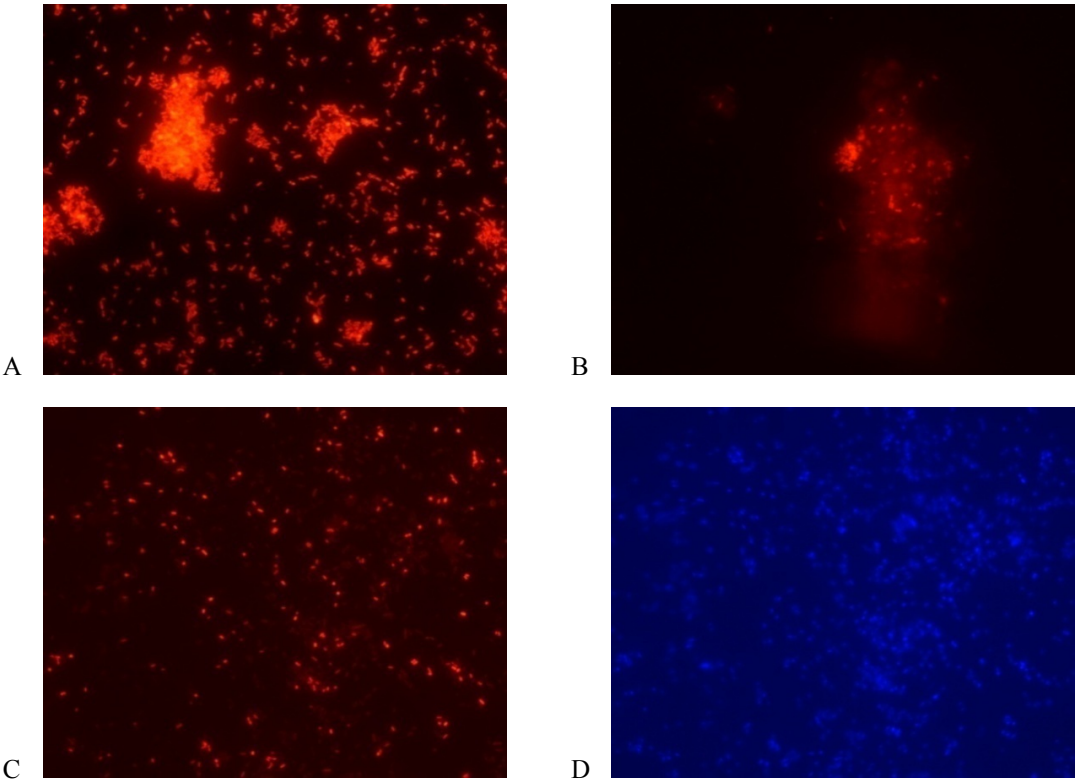

Figure 1 Epifluorescent micrographs of bacteria from marine specimen SK8 with Cy3-labelled (A) EUB338, (B) ALF, (C) CF319, and (D) same microscopic field as in panel A with UV excitation (DAPI staining).

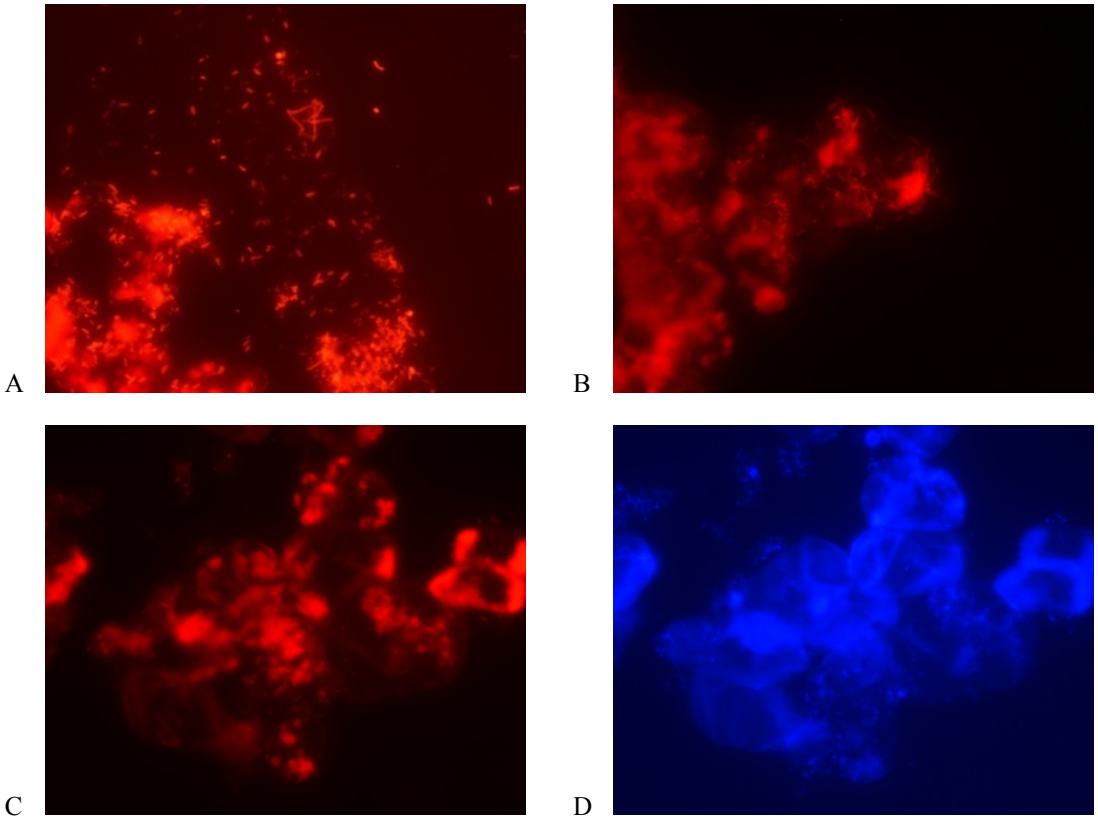

Figure 2 Epifluorescent micrographs of bacteria from marine specimen SK16 with Cy3-labelled (A) EUB338, (B) ALF, (C) CF319, and (D) same microscopic field as in panel A with UV excitation (DAPI staining).

Cytophaga-Flavobacterium group

Members of the *Cytophaga-Flavobacterium* group could be found in some marine specimens and biofilms samples, except for SK9 and SK11, and usually formed the largest bacterial group in the marine specimens. The average total cell counts were 1.96×10^7 to 7.4×10^7 cells ml^{-1} for plant material samples, 1.4×10^7 to 2.94×10^7 cells ml^{-1} for seagrass samples, 1.85×10^7 to 7.2×10^8 cells ml^{-1} for biofilms samples, 4.57×10^8 to 4.96×10^8 cells ml^{-1} for dead fish samples, 3.55×10^7 to 5.8×10^7 cells ml^{-1} for seaweed samples, and 6.6×10^7 cells ml^{-1} for sponge samples.

Alpha-subclass Proteobacteria

The medians of relative abundances and absolute cell numbers of the alpha-subclass *Proteobacteria* detected with the probe ALF1b were significantly higher in the marine samples, except for SK7, SK9, and SK11. The average total cell counts were 8.3×10^5 to 3.6×10^6 cells ml^{-1} for plant material samples, 2.6×10^6 to 9.75×10^7 cells ml^{-1} for seagrass samples, 9.1×10^5 to 3.4×10^6 cells ml^{-1} for biofilms samples, 3.2×10^6 cells ml^{-1} for dead fish samples, 5.49×10^7 to 7.33×10^7 cells ml^{-1} for seaweed samples, and 6.13×10^7 cells ml^{-1} for sponge samples. The morphology of the alpha subclass of *Proteobacteria* was diverse and included rods, vibrios, and filaments of various sizes.

The most abundant phylogenetic group in the Songkhla Lake marine specimens was the *Cytophaga-Flavobacterium* cluster. This is remarkable, since high numbers of *CFB* had so far not been found in marine specimens by either molecular methods [23] or culture-based analysis. Most of the cells identified

within this cluster showed a homogeneous morphology of long, thin rods. Significant numbers of *CFB* members in marine environments have so far been found only in water columns associated with macroscopic marine aggregates or with algal blooms in sea ice [24]. The members of the *CFB* cluster are mainly aerobic, Gram-negative bacteria, specialized for the degradation of complex macromolecules [25,26].

Group-specific probing

When the community composition was further analyzed with a set of oligonucleotide probes targeting larger phylogenetic groups within the domain *Bacteria*, only about 80 % of the cells were detected with probe EUB338. This is certainly due to the incompleteness of the probe set used in this study. New probes must be developed to target sequences found in the rDNA libraries from marine specimens and marine systems [27-29]. In particular, new group-specific probes for the TENA, RAP, AUNA, AUMA, FUL, and CYTO subclasses of the *Cytophaga-Flavobacterium* need to be designed [30].

Throughout the FISH analysis of the marine specimen samples, it was evident that the signal intensities observed for all probe conferred fluorescence were low compared to those of the control cultures. **Figure 3** shows the epifluorescent micrographs of marine gliding bacteria in species of *T. mesophilum*, *Cytophaga* sp., *A. marina*, *R. thailandica*, *A. maritima*, and *F. kasyanovi* with Cy3-labelled CF319. This study used the quantification of FISH data on specific species of marine gliding bacteria. Since this requires relatively high fluorescence intensities, attempts were made to increase the signal intensities of these marine gliding bacteria. One possible reason for the low fluorescence could have been that the fixation protocol was optimized using pure cultures. Another possible reason why the marine gliding bacteria possessed low probe conferred fluorescence was that the cells present in the sample contained low levels of degraded rRNA. Several papers have shown that, in bacteria cultures, there is a link between the growth rate/physiological state of cells, their rRNA contents, and detection of these cells using FISH [31,32].

All probes designed did not have good relative fluorescence hybridization intensities, indicating the high accessibility of each probe to 16S rRNA, based on the relative fluorescence hybridization intensity ratings. The probes TENA, RAP, AUNA, AUMA, FUL, and CYTO had relative fluorescence hybridization intensities of 31 - 50 % (data not shown). Each of the probes was found to hybridize exclusively with the rRNA of the target group under optimum stringency conditions. For all probes, a formamide concentration of 35 % provided sufficient stringency (**Figure 4**). He *et al*. [33] made suggestions for gene-specific and group-specific oligonucleotide probe design. All sequences must have a high identity and a common long stretch within a group. In addition, other criteria, such as GC content, *Tm*, and sequence complexity, also need to be considered. Since GC content varies among different organisms, an oligonucleotide design tool should evaluate all sequences in the data set and determine *Tm* values that fall into a narrow range to ensure quantitative comparison of gene expression. Those parameters should be used as filters for excluding probe candidates.

Using FISH to study marine bacteria composition is particularly useful for observing the development of specific genera or species of marine gliding bacteria; namely, TENA, RAP, AUNA, AUMA, FUL, and CYTO probes for *T. mesphilum*, *R. thailandica*, *A. marina*, *A. maritima*, and *F. kasyanovii* species, and *Cytophaga sp.*, respectively. The microbial communities analyzed were dominated by *Bacteria* (probe EUB338), whereas *Alpha Proteobacteria* (ALF1b) and *Cytophaga-Flavobacterium* (CF319a) were detected only in numbers that remained below the detection limit (**Figure 5**). The total cell counts found in our samples were 10^9 to 10^{11} cells ml^{-1}. Members of the *Cytophaga-Flavobacterium* group found in some marine specimens and biofilms samples, except for SK9 and SK11, were investigated, and usually formed the largest bacterial group in the marine specimens. In *Alpha Proteobacteria*, the medians of relative abundances and absolute cell numbers of the alpha-subclass *Proteobacteria* detected with the probe ALF1b were significantly higher in the marine samples, except for SK7, SK9, and SK11. Additional information on changes in cell size and morphology could be readily obtained. This information is of great ecological importance and allows, for example, the calculation of biomass distribution for defined groups, or the study of the development of marine bacteria populations.

FISH studies with high spatial, temporal, and phylogenetic resolution may considerably increase our knowledge of marine bacteria ecology in the future. In summary, our results showed the 6 new probes for members of CFB-phylum. To our knowledge, this is the first time new probes have been designed specifically *T. mesphilum*, *R. thailandica*, *A. marina*, *A. maritima*, *F. kasyanovii* and *Cytophaga* sp. The new probes do not replace, but supplement, older probes like CF319a, which still remains the most encompassing probe for the CFB-phylum. Moreover, these results indicate the microbial community composition of the marine specimens from Songkhla Lake.

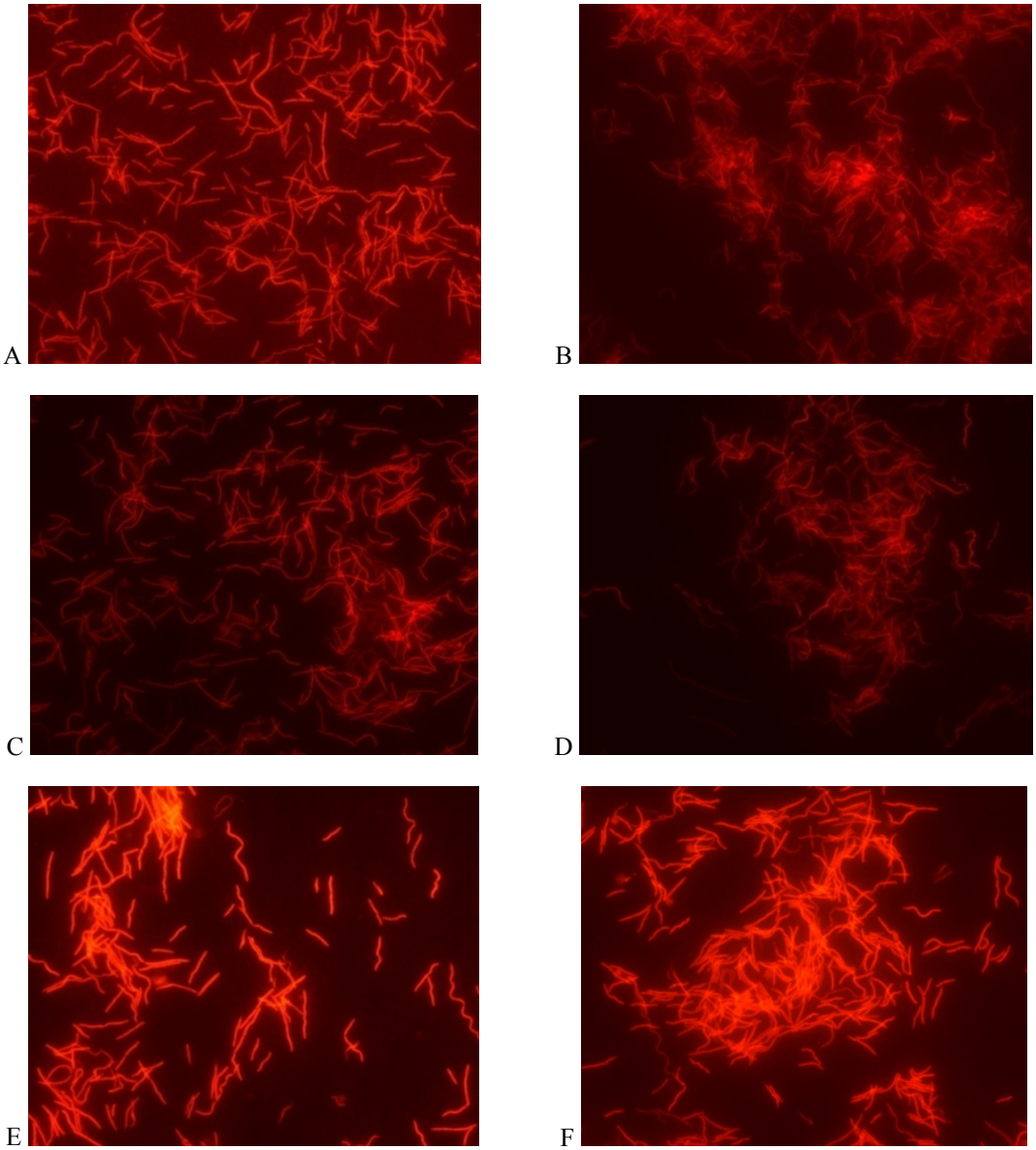

Figure 3 Epifluorescent micrographs of marine gliding bacteria in species of (A) *Tenacibaculum mesophilum*, (B) *Cytophaga* sp., (C) *Aureispira marina*, (D) *Rapidithrix thailandica*, (E) *Aureispira maritima*, and (F) *Fulvivirga kasyanovi* with Cy3-labelled CF319.

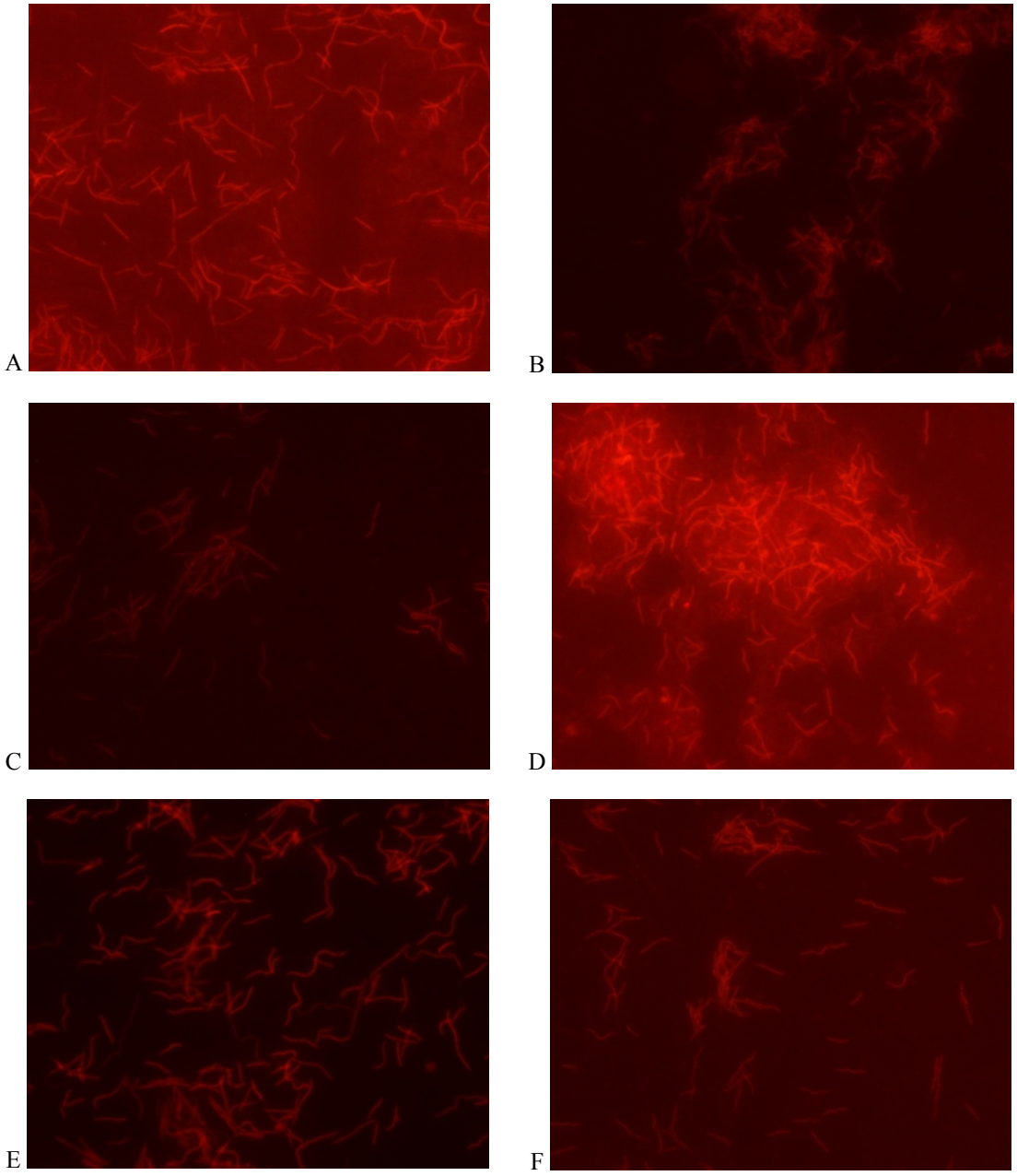

Figure 4 Epifluorescent micrographs of marine gliding bacteria in species of (A) *Tenacibaculum mesophilum*, (B) *Cytophaga sp.*, (C) *Aureispira marina*, (D) *Rapidithrix thailandica*, (E) *Aureispira maritima*, and (F) *Fulvivirga kasyanovi* with Cy3-labelled probe design.

Conclusions

We have added several new probes for members of the CFB-phylum to the set of group-specific probes for the characterization of environmental samples. Their users should always keep in mind that the development of such a set cannot be finished as long as we have only analyzed part of the extant microbial diversity. This study has also been the first to report the design and optimized use of 6 marine gliding FISH probes for marine gliding bacteria of the phylum *Cytophaga-Flavobacterium-Bacteroides*. Future investigations on marine specimens will combine a larger set of specific probes with the analysis of biogeochemical processes to more fully understand the structure and function of marine sediments and specimens. For the microbiologist, analyzing the marine sample process in situ hybridization with probes specific for marine gliding bacteria is a new tool for the rapid and reliable identification of these bacteria in marine specimens. Culture-independent studies may be the key to a better understanding of the principles of marine gliding bacteria or filamentous bulking.

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