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Colonization and community dynamics of class *Flavobacteria* on diatom detritus in experimental mesocosms based on Southern Ocean seawater ☆

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Abstract

In order to better understand the ecology of microorganisms responsible for secondary production in the Southern Ocean the activity of *Flavobacteria* communities on diatom detritus in seawater mesocosms was investigated. Seawater was collected from different parts of the Southern Ocean including the Polar Front Zone (PFZ), ice-edge area of the Antarctic Zone (AZ), and a site in the AZ ice pack. Detritus from the cosmopolitan marine diatom *Nitzschia closterium* Ehrenberg was resuspended in mesocosms containing seawater filtered to remove particulate organic matter, including particle-associated bacteria and most eukaryotes, but retaining native planktonic bacterial assemblages. Mesocosms were incubated at 2 °C and samples analysed for changes in community composition using denaturing gradient gel electrophoresis (DGGE), real-time PCR and fluorescent in-situ hybridization (FISH). DGGE banding patterns and FISH images demonstrated rapid bacterial colonization of the detritus, dominated by members of class γ-*Proteobacteria*, α-*Proteobacteria* and *Flavobacteria*. Real-time PCR data indicated members of class *Flavobacteria* were involved in initial colonization of detrital aggregate, however relative abundance stayed at similar levels found for the original native particle-associated populations. 16S rRNA gene DGGE banding patterns and sequence analysis demonstrated significant variation in Flavobacteria community structure occurred in the first 20 days of the experiment before community stabilization occurred. The community structures between the three mesocosms also markedly differed and major colonizers were primarily derived from detectable members of the initial particle-associated Flavobacteria community, however the abundant uncultured Flavobacteria agg58 clone-related and DE cluster 2 clades, previously identified in Southern Ocean seawater were not observed to colonize the detritus.

Keywords: Seawater; Southern Ocean; DGGE; *Flavobacteria*; *Cytophaga-Flavobacterium-Bacteroides* group; Biogeography; Psychrophilic bacteria

1. Introduction

Algal phytoplankters, in particular diatoms, make the largest contribution to primary production in the Southern Ocean euphotic zone [1]. Silica, nitrate and phosphate levels in parts of the Southern Ocean are often very low in summer, nearly exhausted due to bursts of new phytoplankton production [2]. Nutrients for new production are mainly supplied by decomposition of existing biomass, especially in areas far from continental landmasses. Indeed, microbial secondary production mineralizes about one-half of the primary produced biomass in the oceans [3]. Secondary production is thus a critical mechanism in the maintenance of marine foodwebs. More knowledge is required to understand the

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ecology of microbially driven secondary production processes [4].

Previous studies aimed at examining bacterial mediated degradation of phytoplanktonic biomass have demonstrated bacteria accelerate biogenic silica dissolution [5]. Examination of silica cycling through the application of mesocosms has been used to examine specific mechanisms for microbial secondary production including silica redispersal [6,7]. The use of experimental enclosures to examine the dynamics of environmental processes have been utilised to describe a number of other microbial processes and ecological relationships, including bacterial activity during diatom blooms, the effects of variations in nutrient composition on bacterial communities, and bacterial motility [8–14].

The ability to examine the response of a community to simple and distinct changes such as nutrients, temperature, salinity or grazing allows reduction of the variables and potentially permits clearer evaluation of specific processes. In this way, it is possible to assign specific characteristics or functional relationships to microbial communities or subgroups. The use of mesocosms, like many experimental approaches involves certain biases; in the proposed diatom detritus-based mesocosms studied here it is likely a treatment effect will manifest in which the provided detrital surfaces will result in abnormally enhanced bacterial activity and populations [15]. Despite the possible influences of such an effect on the community structures obtained, the mesocosm studies provide an opportunity to study specific processes through the elimination of complicating variables present in the marine environment.

The study was aimed at examining the response of native Southern Ocean bacterial communities, specifically the *Flavobacteria*, to the addition of diatom detritus. *Flavobacteria* were studied as they represent an important component of the bacterioplankton in the Southern Ocean seawater and sea-ice zones [16–20]. In general, members of the CFB phylum can be potentially dominant components of seawater microbial communities either on marine aggregates [21,22] and/or in the planktonic phase [23] and are exceptionally responsive to phytoplanktonic blooms [24]. The group plays a significant role in the degradation of organic matter, particularly the high molecular weight fraction in seawater, including proteins, polysaccharides and diatom debris [11,25–27]. Biogeographic information indicates that Flavobacteria communities in the Southern Ocean statistically differed between water masses north and south of the Polar Front with diversity and particle-associated abundance substantially enhanced in the Antarctic Zone (AZ) relative to nutrient-limited waters of the Sub-antarctic Zone (SAZ) [20]. Denaturing gradient gel electrophoresis (DGGE) gel band sequence data indicated CFB encompassed substantial diversity with several uncultivated clades spread throughout class *Flavobacteria*. Based on data from diversity studies using bacteria-specific primers class *Flavobacteria* made up the vast majority of the CFB diversity in Southern Oceans waters [20]. Class *Flavobacteria* consists of family *Flavobacteriaceae* and the newly created family *Cryomorphacaeae* [28], which incorporates the agg58 clone cluster, a predominantly uncultured clade that has been identified as a common inhabitant of seawater algal blooms [22,29,30]. Members of family *Flavobacteriaceae* have been clearly shown to be predominant in colonization of natural and experimental phytoplanktonic blooms as well as in decomposition experiments [24]. Recent data also indicates the *Flavobacteriaceae* predominates in Southern Ocean seawater, particularly in the Polar Front and Antarctic Zones between latitudes 58–68 ° S [20] and within sea-ice algal assemblages [19].

In this study, mesocosm studies examining the colonization of diatom detritus by planktonic bacteria from Southern Ocean surface waters reveals that many *Flavobacteria* taxa that were derived from already partially decomposed particulate organic matter exhibit a pronounced alacrity for phytoplankton biomass colonization.

2. Methods

2.1. Preparation of diatom detritus

An axenic strain of *Nitzschia closterium* Ehrenberg was obtained from the CSIRO Collection of Living Microalgae and was cultured in F/2 medium in continuous light (250 μmol photons m^{-2} s^{-1}) at 20 °C with continuous aeration and mixing. F/2 medium consisted of 75 mg $NaNO_3$, 5.65 mg $NaH_2PO_4 \cdot 2H_2O$, 1 ml trace metal stock solution and 1 ml vitamin solution in 1000 ml natural seawater, pH 8.0. The trace metal solution consisted of 4.36 g disodium EDTA, 3.15 g $FeCl_3 \cdot 6H_2O$, 10 mg $CuSO_4 \cdot 5H_2O$, 22 mg $ZnSO_4 \cdot 7H_2O$, 10 mg $CoCl_2 \cdot 6H_2O$, 0.18 g $MnCl_2 \cdot 4H_2O$ and 6 mg $Na_2MoO_4 \cdot 2H_2O$ in 1 l distilled water. The vitamin solution consisted of 0.5 mg cyanocobalamin, 0.1 g thiamine HCl and 0.5 mg biotin in 1 l distilled water. The final culture was spun down at 1000*g* in 200 ml Nalgene R centrifuge containers and resuspended in filtered artificial seawater (FASW, 35 $g\,l^{-1}$ sea salts, Aquasonic, Wauchope, NSW, Australia). Diatom detritus was created from cell suspensions using freeze–thaw [6]. Following this treatment detritus was washed (4000*g*, 10 min) and resuspended in FASW.

2.2. Mesocosm setup

Native free-living bacterioplankton communities were obtained by filtering three different seawater sam-

ples, S1, S2, and S3, onboard the *RV Aurora Australis* through a 0.8 μm capsule filter (Pall Gelman, Ann Arbor, MI, USA) to remove all particulate matter [20]. Sample S1 was obtained from the Polar Front Zone (53 54° S, 141 21° E, water temperature 2.4 °C), S2 from the ice edge zone (60 44° S, 139 54° E, temperature 0.1 °C) and S3 from within the ice pack (63 26° S, 144 05° E, temperature −1.1 °C). The filtrates collected nearly completely filled 25 l carboys; 5 mg l^{-1} of diatom detritus was added to each of the carboys at time zero (T0), which were stored with gentle aeration at 2 °C. For DNA extraction, approximately 300 ml of water was sampled from each carboy and filtered through a 0.8 m polycarbonate filter (Millipore). The filters were washed by filtering through 50 ml of FASW to remove loosely associated and free living bacteria and then frozen. For fluorescent in-situ hybridization (FISH), two 50 ml samples were obtained from each carboy and filtered through 0.8 μm filters. Filters were then fixed, air-dried and stored at room temperature [16]. For each of the three mesocosm experiments controls were also tested in parallel. Control mesocosms contained filtered autoclaved seawater and diatom detritus.

2.3. Dissolved silica analysis

Samples (3×10 ml) taken from carboys for silica analysis at were filtered with 0.5 μm pore size cellulose nitrate filters and the filtrate was then aliquoted into two 12 ml nutrient analysis tubes, and frozen. Silica analysis was later performed using the molybdate reduction [31] procedure with samples compared to a standard curve prepared with $Na_2SiO_3 \cdot 5H_2O$ dissolved in distilled water.

2.4. Fluorescent in situ hybridization

Filters with fixed cellular material were stained with oligonucleotide probes as previously described [16]. Cy3-labelled oligonucleotide probes (Geneworks, Australia) used included Eub338 (Bacteria), Alf968 (α-*Proteobacteria*), Gamm42a (γ-*Proteobacteria*) [16] and Flavo1 (class *Flavobacteria*) [32]. Following hybridization, filters were washed and stained for 15 min at 48 °C on a heating block in 5 ml of wash buffer [32] containing 2 μg ml^{-1} 4′, 6-diamidino-2-phenylindole (DAPI). The filters were dried and mounted on a glass slide with immersion oil and inspected, with digital pictures taken using a LDRMBE Leitz microscope (Leica, Heerbruug, Switzerland) fitted with a DC300F digital camera (Leica), equipped with a 50-W HBO mercury bulb (Osram, Regensburg, Germany) and specific filter sets for DAPI and Cy3 fluorophores. Fields were first viewed with the Cy3 filters before switching to the DAPI filters, in order to minimize photo-bleaching of the Cy3 fluorophore during DAPI examination. Cell populations in the planktonic phase were measured by DAPI direct counting [10].

2.5. Mesocosm DNA extraction and purification

Extraction of DNA from filter samples followed a modification of a method employed by Fuhrman et al. [33] as described by Abell and Bowman [20].

2.6. Real-time PCR

Real-Time PCR reactions were prepared with the QuantiTect SYBR Green PCR kit (Qiagen, USA) in 200 μl flat lid PCR tubes (Axygen Scientific, Union City, USA) and used the Rotorgene thermocyler (Corbett Research, Australia) [20]. Results were analysed with Rotorgene software (V. 4.6). Primers used for amplification and detection of class *Flavobacteria* 16S rRNA genes included 558f (5′-ATT GGG TTT AAA GGG TCC-3′) and 907r (5′-CCG TCA ATT CCT TTG AGT TT-3′) [34]. This primer pair has been found to be highly specific for class *Flavobacteria* [20]. Total bacterial 16S rRNA gene abundances were estimated with primers 519f (5′ CAG CMG CCG CGG TAA TAC 3′) [34] and 907r. Assays were performed using a thermocycling program consisting of an initial 15 min 95 °C step followed by 35 cycles consisting of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s with fluorescent acquisition, and a further fluorescent acquisition step at 80 °C. Positive control standards for the real-time PCR assay included 16S rRNA genes amplified with primers 558f/907r from *Gelidibacter algens* and *Cryomorpha ignava*. The controls were diluted in tenfold series from 1 ng to 0.1 pg and analysed in parallel with seawater DNA samples. Negative controls included samples lacking template DNA and samples lacking primers. Experimental samples were diluted to three different concentrations and compared to the standard curve ($r^2 > 0.99$) in the same run. Results were considered only if PCR efficiency was at least 0.8. Values were corrected for their dilution factor and averaged to calculate the total 16S rRNA amount in the starting material and then compared to the same sample analysed with the *Flavobacteria* specific primers to give a percent fraction of class *Flavobacteria* 16S rRNA to total bacterial 16S rRNA.

2.7. Denaturing gradient gel electrophoresis

A class *Flavobacteria* specific 16S rRNA gene primer set, 558f and 1392rc (5′-CGC CCG CCG CGC CCC CGC CCG GCC CGC CGC CCC CGC CCC ACG GGC GGT GTG TAC-3′) [35], were used to amplify regions of the 16S rRNA gene as described by Abell and Bowman [20]. DGGE was performed using the D-code Universal Mutation Detection System (Bio-Rad

Laboratories, Hercules, USA) using parallel 6% polyacrylamide gels containing a 40–60% denaturing gradient, with 25 μl of PCR product (quantified by fluorometry with Hoechst dye 33258) and 5 μl of gel loading dye loaded onto gels, operated at 60 °C for 16 h. Gels were stained with Sybr-gold nucleic acid stain (Molecular Probes, Eugene, USA) and imaged using a UV transilluminator equipped with the MP4+ Instant Camera System (Polaroid, Waltham, USA) with an attached Sybr gold filter. In order to reduce variation between individual gel runs, PCR and DGGE were performed in triplicate for all samples and the data pooled to assess gel variation [36]. Banding patterns and band intensities were found to be consistent between replicates. DNA band extraction, reamplification and sequence analysis followed a previously described protocol [20]. Seawater samples used in the mesocosms, with no added diatom detritus were stored in 1 l Schott bottles at 2 °C were also examined at T0, T10 and T30 by DGGE to assess variation in the seawater community over the duration of the mesocosm experiment. DGGE band profiles were analysed by multivariate statistics with the Diversity Database (v. 2.2.0, Biorad) and Primer 5 (v. 5.2.4., Plymouth Marine Laboratories, UK; www.pml.ac.uk/primer) programs, using the non-metric multidimensional scaling (nMDS) and analysis of similarity (ANOSIM) methods as previously described [20,36].

2.8. Bacterial isolation and identification

Bacterial isolation was conducted on Southern Ocean surface seawater particulate material collected throughout the PFZ and AZ [20] using plankton net (20 μm sized mesh) tows. Samples from the plankton nets were suspended in sterile filtered seawater and plated within 1 h onto Seawater Nutrient Agar [29]; Marine Agar (Difco Laboratories, Detroit, Mich., USA); and SFA agar [37] followed by incubation at 2–4 °C for up to 12 weeks. Colonies chosen for further analyses were selected based on differing colonial morphotypes and purified on fresh media for subsequent identification. Genomic DNA was extracted from cells and purified using the Marmur protocol [38]. The 16S rRNA gene from each of the isolates was amplified by PCR and sequenced as previously described [29].

2.9. 16S rRNA gene sequencing

Sequence data was viewed and checked using the BioEdit Sequence Alignment Editor [39]. The 16S rRNA gene sequence for each strain was compared to the sequences in the Genbank nucleotide database using the BLAST search program of the National Center for Biotechnology Information website http://www.ncbi.nlm.nih.gov. Sequences were aligned to downloaded reference sequences using the Bioedit sequence alignment editor. Analysis of the resulting 16S rRNA gene sequence datasets was performed using the PHYLIP suite of programs [40]. Evolutionary distances were determined using the Maximum likelihood algorithm in the program DNADIST and phylogenetic trees were constructed using the neighbor-joining method using the program NEIGHBOR. The programs SEQBOOT and CONSENSE were used for bootstrap analysis, utilizing 1000 replicates. The 16S rRNA gene sequences obtained in this study were deposited in the Genbank nucleotide database under the accession numbers AY285940-285949, AY298788, AY319330, AY353812-353822, AY533182-533199, and AY661567-661610.

3. Results

3.1. Mesocosm silica dissolution

Analysis of dissolved silica (dSi) concentrations in each of the seawater mesocosms (S1, S2, and S3) demonstrated an increase in dSi concentrations above the corresponding controls. The controls reached a final dSi concentration of between 1.15 and 1.76 mg l^{-1}, by comparison the mesocosms reached final dSi concentrations of between 3.2 and 4 mg l^{-1} (Fig. 1). The rate of increase of dSi above that measured in the control mesocosm varied between mesocosms, with mesocosms S1 and S2 demonstrating a significant increase above the control mesocosm after 5 days, while mesocosm S3 failed to demonstrate a significant increase until 10 days had passed. A biphasic response was evident in S1 and S3 with accelerated dissolution appearing to occur in the latter stages of the experiment (Fig. 1).

3.2. Microscopic examination of detrital particles by FISH

Planktonic bacterial DAPI direct counts in the three mesocosms were similar and increased from 0.9–1.4×10^6 to 3.2–5.6×10^6 cells l^{-1} between day 0 and day 10. By days 20–30, the population range had stabilized to a mean of 6.0×10^6 cells l^{-1}. Particulate matter in the mesocosms was primarily comprised of aggregated diatom frustules that coalesced within the first 24 h of the experiment in all of the mesocosms. FISH was not used for accurate quantification of bacterial numbers as early detrital samples produced intense background autofluorescence or were too densely clustered on particles, making the counting of colonizing bacteria extremely difficult. Nevertheless, the use of FISH confirmed the colonization of detritus by bacteria, demonstrated the presence of members of the *Flavobacteria* attached to the diatom detritus and clearly indicated that a high density of colonizers attached to the diatom detritus in the latter stages of the experiment

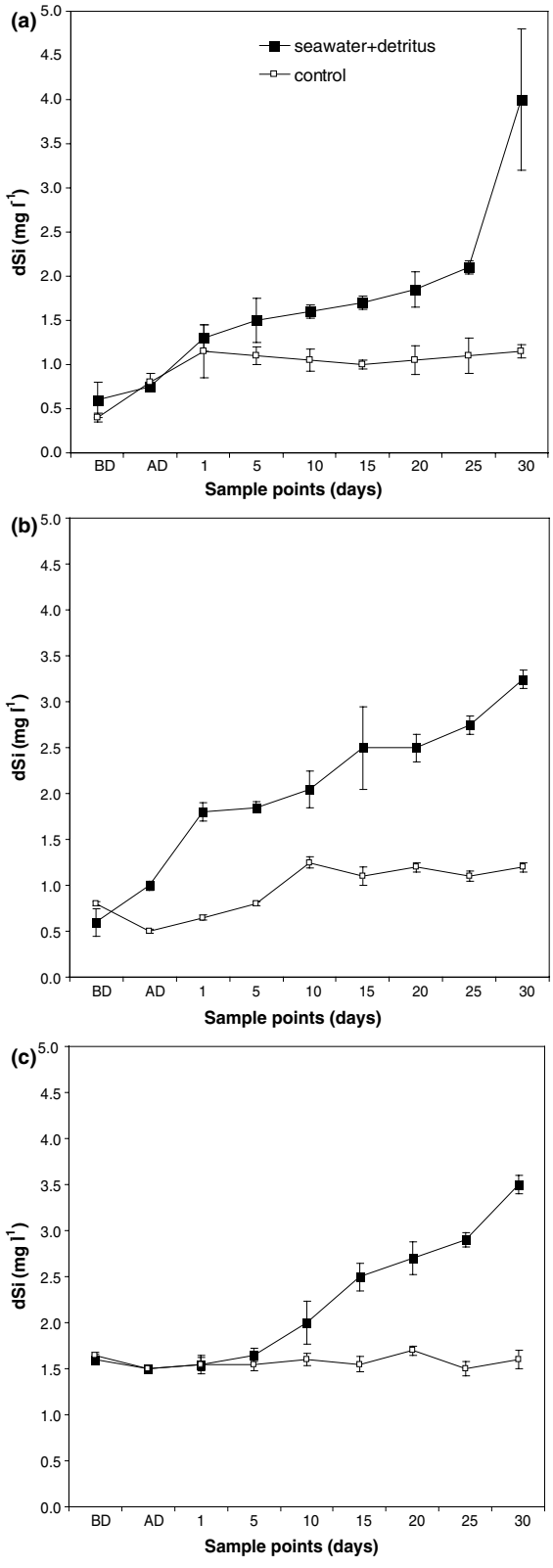

Fig. 1. Dissolved silica in mesocosms S1, S2 and S3 containing native bacterial populations and control mesocosms over the 30 days of incubation. Time points "BD" and "AD" represent silica values before (BD) and after (AD) the addition of diatom detritus (at T0), respectively.

(Fig. 2). By day 20 >95% of the DAPI count consisted of members of the α-*Proteobacteria*, γ-*Proteobacteria* and *Flavobacteria*. γ-*Proteobacteria* dominated the detrital populations in mesocosms S2 (Fig. 2) and S3.

3.3. *Real-time PCR quantification of* Flavobacteria *colonizing diatom detritus*

Analysis of *Flavobacteria* colonization of detritus utilized the real-time PCR technique and the seawater samples used in the mesocosm experiments all contained particulates, captured on 0.8 μm filters, that had been colonized by *Flavobacteria* (T0, Fig 3). The relative abundance varied 4-fold between the samples, with mesocosm S1 possessing a high relative abundance of *Flavobacteria* in the particulate fraction (29% of total 16S rDNA). Colonization of the added bacteria-free detritus in mesocosm S1 was rapidly dominated by members of the *Flavobacteria*, reaching a relative abundance of greater than 70% by day 15 before declining to 30–45% between day 15 and day 30. The other two mesocosms (S2 and S3) demonstrated much lower *Flavobacteria* abundance in the initial detritus colonization period (Fig. 3). Over time the abundance increased in proportion to the total bacterial abundance though the extent of this differed considerably between mesocosms. In mesocosm S2 *Flavobacteria* colonization resulted in abundances slightly exceeding the original particulate level, ranging from 7% to 15% from day 15 to day 30 of the experiment. For mesocosm S3 the abundance between day 15 and day 30 only ranged from 1% to 5%, lower than the 17% abundance present in the original particulate fraction. Overall, the data suggested that in the case of mesocosm S3 the original *Flavobacteria* population seemed to had been displaced by other varieties of bacteria, however *Flavobacteria* were comparatively more successful in the other mesocosms.

3.4. *DGGE analysis of* Flavobacteria *colonizing detritus*

Examination of DGGE profiles demonstrated that the majority of *Flavobacteria* phylotypes colonizing the diatom detritus particles were present as major communities in the native seawater attached-fraction. This was initially suggested by DGGE bands from mesocosm samples corresponding to the same position of bands obtained in profiles of the original particulate-associated communities (Fig. 4). This was confirmed by sequence analysis, which indicated corresponding DGGE bands represented the identical 16S rRNA gene phylotype (Table 1). DGGE band profiles also indicated that the initial particle associated *Flavobacteria* community structures were initially different between the seawater samples S1, S2 and S3 (Fig. 4).

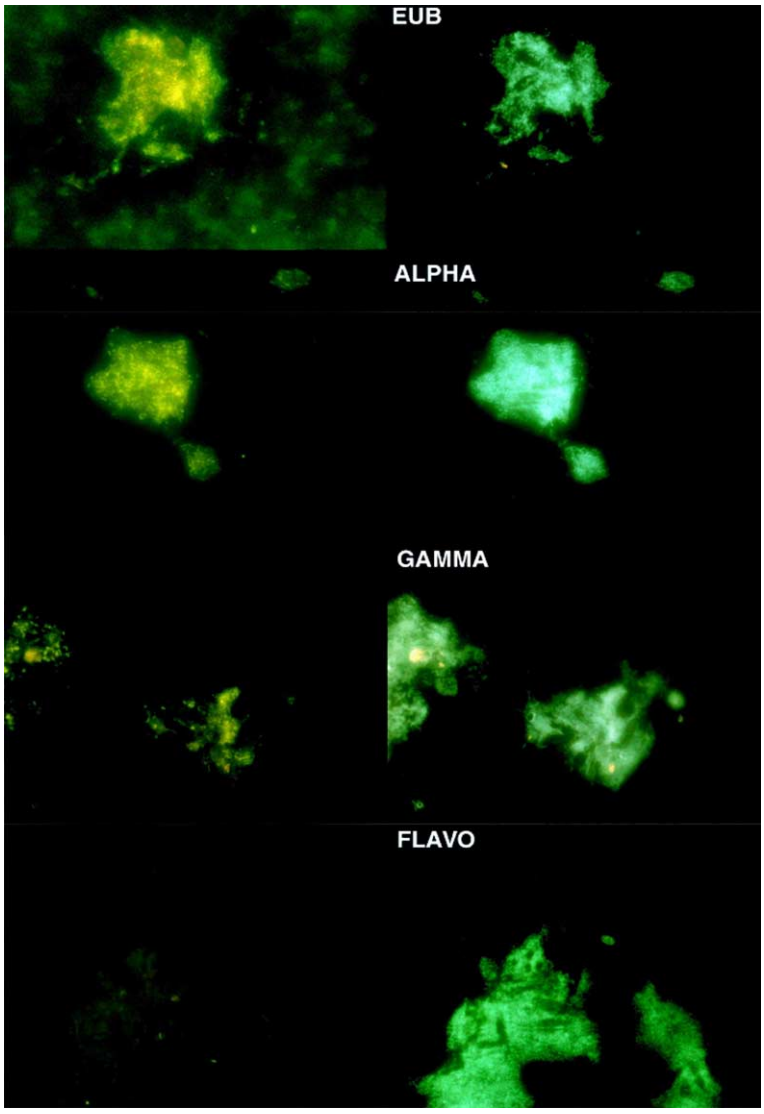

Fig. 2. Epifluorescent micrographs of detritus particles using FISH to demonstrate colonization by different groups. The left panel demonstrates cells hybridized to the corresponding probe, whilst the right panel demonstrates total (DAPI stained) cells. All samples are from day 20 of mesocosm S2.

The DGGE profiles revealed that colonization was rapid. In the S1 mesocosm bands were faintly present after 1 d but became clearly obvious after 5 days incubation. Band intensity varied for some but not other bands in S1 (e.g., bands at T5 compared to those at T10) suggesting different species in the mesocosms may have changed in population substantially during the early to mid-phases of the experiment before eventually establishing a more stable community structure after about 20 days of incubation. In the case of mesocosms S2 and S3 DGGE bands became only apparent after 10 days incubation and like mesocosm S1 attained a stable community structure after 20 days (Fig. 4). The DGGE profiles arising from the diatom addition differed starkly with seawater samples containing no added detritus after a periods of 10 and 30 d. The seawater community were found to be relatively stable when stored at 2 °C with no new bands appearing and producing profiles similar to that found for T0. nMDS and ANOSIM analysis (methods described in [20,36]) of these patterns revealed there was no statistical difference ($p > 0.05$) between the T0, T10 and T30 sampling time intervals. By comparison, the profiles between seawater samples S1, S2 and S3 and in samples subsequently amended with diatom detritus were significantly different ($R \geqslant 0.90$, $p \leqslant 0.001$) to each other throughout the entire course of the experiment.

The DGGE profile patterns obtained for S1 appeared to closely reflect that of the original community over the course of the experiment, however two bands (indicated as numbers 105 and 108, Fig. 4) were not detected in the original particulate community at T0. Instead the bands only became detectable after 20 d incubation. In mesocosms S2 and S3 by comparison the colonization of

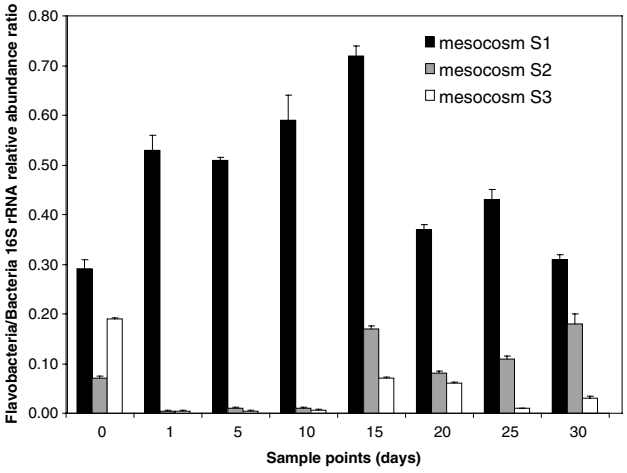

Fig. 3. Real-time PCR analysis results of samples from diatom detritus amended seawater mesocosms, measuring the relative abundance of class *Flavobacteria* (indicated as a proportion of the total 16S rRNA gene abundance) over the course of the experiment (T0 to T30, days of incubation).

the diatom detritus yielded substantially simpler communities by the end of the experiment. This was especially the case for mesocosm S3 where the initial, relatively complex Flavobacteria community (~20 visible bands) was reduced to few detectable species (4 or 5 obvious bands; Fig. 4). Based on band intensity these few species were considerably favored (e.g., DGGE band 1 in mesocosm S3) in the mesocosms at the expense of other, potentially more abundant species.

3.5. Analysis of DGGE band phylotypes

Sequencing of phylotypes detected using DGGE, demonstrated that the members of the *Flavobacteria* colonizing the detritus belonged mostly to cultured genera (Table 1, Fig. 5), including *Polaribacter*, *Tenacibaculum*, *Zobellia*, *Psychroserpens*, *Aequorivita* and the newly described genera *Biziolla* and *Subsaximicrobium* [41]. However, two phylotypes (S104a and S205) demonstrated little homology (<96%) with known species or clones. Mesocosm S1 had the greatest diversity of phylotypes colonizing the diatom detritus including representatives of 8 different genera. Except for bands S105 and S108 (Fig. 5) all were present on the original seawater particulates. The colonization of diatom detritus in mesocosm 2 was dominated by *Tenacibaculum* species (S201, S202, S203) and also included a *Polaribacter* sp. (S204). At the 25 d incubation point band S205 appeared and rapidly increased in detectability by day 30. Interestingly, band S205 was also prominently present in the original particulate community. In mesocosm S3 the *Flavobacteria* that colonized the diatom detritus was dominated completely by *Polaribacter* species.

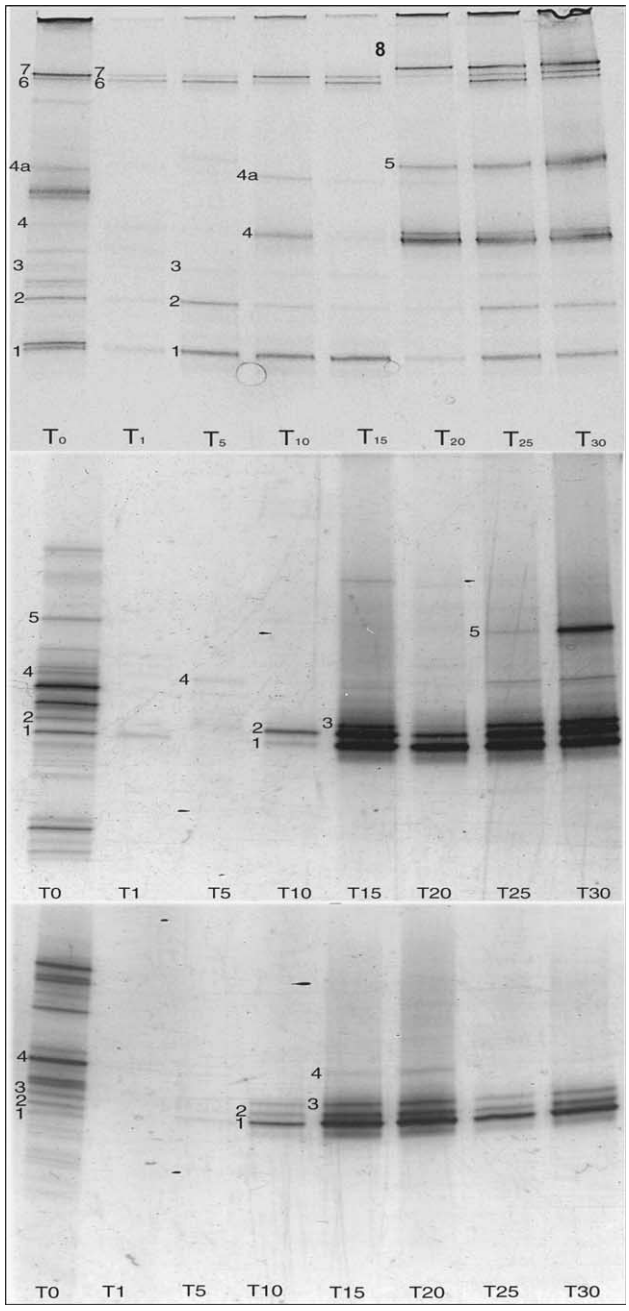

Fig. 4. DGGE gels of the mesocosms experiments demonstrating the succession of *Flavobacteria* phylotypes. Band numbers correspond to phylotypes shown in Table 1. Mescosm S1 (top gel), S2 (middle gel) and S3 (bottom gel). The profile at T0 is derived from particle associated bacteria filtered from the original seawater sample.

3.6. Culture-based survey

Isolates obtained from seawater particulates, segregated into twenty-three colony morphotypes, were identified as taxa belonging to the family *Flavobacteriaceae*. Most isolates belonged to either the genera *Polaribacter* or *Tenacibaculum* (Fig. 5). Two isolates belonged to the genus *Psychroserpens* while another three isolates

Table 1
Phylogenetic affiliation of CFB phylum related bacteria colonizing diatom detritus, detected using DGGE

Mesocosm	DGGE band No.	GenBank No.	Microcosm detection (days)[a]	Closest cultured species/strain[b]	Sequence similarity (%)
S1	S101	AY533182	1–30	*Maribacter sedimenticola*	99
S1	S102	AY533183	1–30	*Aequorivita antarctica*	100
S1	S103	AY533184	5–30	"*Subsaximicrobium wynnwilliamsii* "	99
S1	S104	AY533185	10–30	*Tenacibaculum* sp. strain G1A1	100
S1	S104a	AY533186	10–30	Strain Gaa204net2-3	97
S1	S105	AY533187	20–30	*Flavobacterium gelidilacus*	98
S1	S106	AY533188	1–30	*Formosa algae*	97
S1	S107	AY533189	1–30	*Psychroserpens* sp. strain G512M1	100
S1	S108	AY533190	20–30	*Formosa* algae	96
S2	S201	AY533191	1–30	*Tenacibaculum* sp. strain G121102s2_3	100
S2	S202	AY533192	10–30	*Tenacibaculum* sp. strain G11A2	100
S2	S203	AY533193	15–30	*Tenacibaculum* sp. strain G11A2	99
S2	S204	AY533194	5–30	*Polaribacter* sp. strain G912S3A	99
S2	S205	AY533195	25–30	*Algibacter lectus*	94
S3	S301	AY533196	5–30	*Polaribacter* sp. strain G812S3	100
S3	S302	AY533197	10–30	*Polaribacter* sp. strain G1712M2	98
S3	S303	AY533198	5–30	*Polaribacter* sp. G1612M2	98
S3	S304	AY533199	15–20	*Polaribacter* sp. strain G812S3	100

[a] Mesocosm sampling times over which the DGGE band phylotype was detectable.
[b] Species and strain listed are shown on Fig. 4.

(strains G1A11, Gaa0204net2_3, G812M2) had sequence similarities of less than 92% to their closest cultured representatives and thus represented novel genus-level lineages. Strain G1A11 was equivalent to clade IX in the study of Abell and Bowman [20].

Many non-Flavobacteria strains were also obtained and these were identified as belonging (or related to) the genera *Psychrobacter*, *Marinobacter*, *Pseudomonas*, *Colwellia*, *Thalassomonas*, *Vibrio*, *Glaciecola*, *Pseudoalteromonas*, *Sphingomonas*, *Erythrobacter*, *Maricaulis*, *Hyphomonas*, *Roseovarius*, *Sulfitobacter*, *Micrococcus*, *Frigoribacterium*, *Nocardioides* and *Bacillus* (data not shown). The 16S rRNA gene sequences of these strains are deposited under GenBank accession numbers AY661567-661610.

4. Discussion

DGGE analysis of mesocosm communities using a size fractionation approach has proven to be reproducible and the carry over of bacteria not associated with particulate material appears relatively small [8,12]. This was suggested by the presence of at most very faint DGGE bands being detected in the initial 24 h of the mesocosm experiments (Fig. 4). The faint bands may be due to some large planktonic cells or aggregates of small cells being trapped on the 0.8 μm pore-sized filters. DGGE profiles were found to be reproducible for samples obtained during the duration of the experiments, indicating biases caused by small variations in PCR and electrophoretic running conditions were not significant. It is important to note that possible stochastic variation occurring within individual mesocosm experiments was not assessed so it is uncertain whether similar communities could have been repeatedly and consistently obtained with the same water samples. Limitations in DGGE analysis in regards to seawater mesocosm studies have been discussed previously [8,42]. It has been considered methodological biases if occurring were consistent but more importantly it was recognized that DGGE is unable to comprehensively analyze the entire microbial community. One way to overcome this is to focus on a particular group or clade, increasing the effective resolution of the analysis, allowing for more accurate assessment of banding patterns and more reliable band sequence acquisition. Studies examining the entire bacterial community using the DGGE approach usually can only gain a partial resolution of complex communities and the denaturant gradient used may also promote multiple phylotypes to occur in a single band. This problem worsens considerably in ecosystems that have very high diversity, such as sediments and soils [27,36]. DGGE-PCR sensitivity is also an issue as increasing gene target numbers limits the effective resolution with only the most common phylotypes obtained. This in turn can affect the sensitivity limit, which for DGGE-PCR has been estimated to be at about 1000–5000 cells ml^{-1} [43,44]. This means species detected by DGGE-PCR in the original seawater and mesocosm samples typically represent at least 0.1% of the bacterial population. Importantly this means there could be many initially undetectable taxa capable responding to changing conditions and forming a DGGE band through growth. By focusing on a single group a higher proportion of active taxa present in a sample can be detected providing a clearer insight into some of the dynamics that may be occurring in the experimental system.

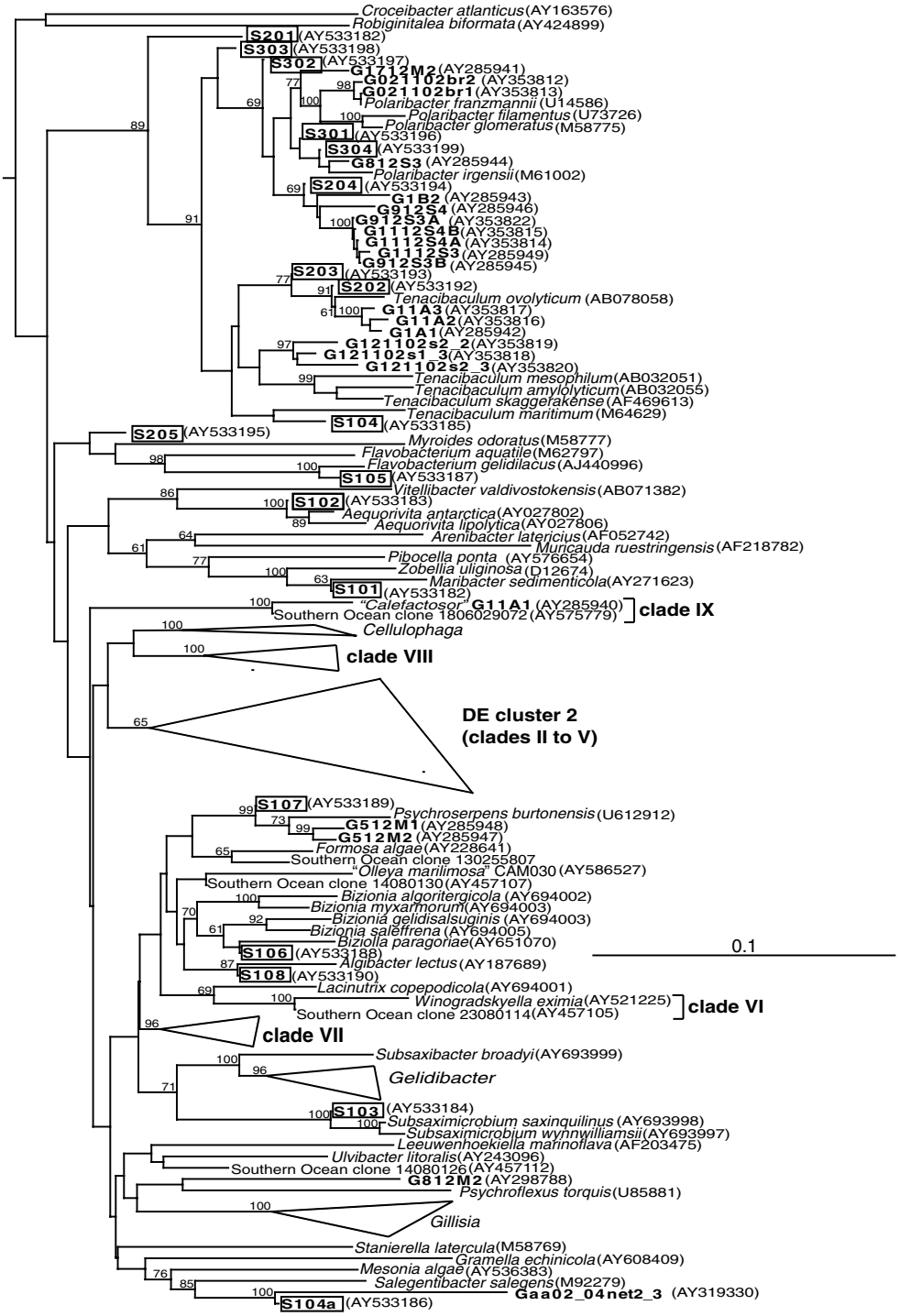

Fig. 5. 16S rRNA gene phylogenetic tree showing the position of bacteria affiliated with family *Flavobacteriaceae* colonizing diatom detritus in the mesocosm experiments (bold, enclosed in boxes) and seawater isolates (bold type). Clade groups indicated were defined in the study Abell and Bowman [20]. *Rhodothermus marinus* and *Chlorobium limicola* were the outgroup sequence. Branch nodes with >60% supporting bootstrap values (500 replicates) are shown only. The scale bar indicates 0.1 changes per nucleotide.

In various phytoplankton bloom and decomposition based seawater experimental systems [8,12,23] members of the family *Flavobacteriaceae* are clearly a predominating presence [24]. Other groups found, include the agg58 (family Cryomorphaceae) and *Lewinella*/agg32 clades (family "Saprospiraceae"). This is completely congruent with the sorts CFB detected using Bacteria-specific primers in the Southern Ocean [19,20] and elsewhere [22,29]. The distribution of phylotypes found in the mesocosms in this study grouped entirely in the family

Flavobacteriaceae; agg58 clade members were not detected. Members of the family *Saprospiraceae* would not have been detected since this group falls outside of class *Flavobacteria* and cannot be amplified with the PCR primers used [20]. The fact that each mesocosm supported a different community of *Flavobacteria* is indicative of the remarkable diversity of the *Flavobacteria* in the Southern Ocean [19,20] and apparently other oceanic regions [22].

Tenacibaculum spp. were common to S1 and S2 mesocosms suggesting these bacteria may be unusually responsive to diatom detritus. The genus *Tenacibaculum* was not detected in detailed molecular surveys of Southern Ocean seawater and sea-ice [19,20] but appear readily cultivable from seawater particulates (Section 3.6), which may suggest the detectable populations of *Tenacibaculum* could be highly transient, growing only to significant populations as attached populations or in association with detrital organic matter. During summer Southern Ocean sea-ice thaw algal material and dissolved organic carbon is released but still can be trapped between ice floes results in an enriched ($>10^9$ cells l^{-1}), highly active but transient bacterial community due to large amounts of organic matter [45]. Mesocosms used in this study are in some ways similar to this scenario as a high concentration of detrital material (5 mg l^{-1}) is suddenly introduced to seawater. It is likely a much lower initial amount of added diatom detritus may result in different responses amongst members of class *Flavobacteria* due to the lower amounts of degradable organic matter.

Active microbial detritus decomposition was clearly indicated by increases in dSi (Fig. 1). This increase corresponded to increases in bacterial numbers present on the detrital particles as was also suggested by the DGGE-PCR profiles in which numerous bands rapidly appeared over time (Fig. 4). DAPI direct counts of planktonic cells increased approximately 5-fold before stabilizing. FISH-based observations indicated that members of the α-*Proteobacteria*, γ-*Proteobacteria* and *Flavobacteria* eventually densely populated the detrital material. Supplementing this information, real-time PCR data showed that *Flavobacteria* colonized detritus in all of the mesocosms (Fig. 3). Moreover, the majority of the *Flavobacteria* phylotypes colonizing the detritus were present in the original particulate fraction of the seawater samples from which the mesocosms were based.

Though the particulate fraction was removed from the seawater samples before addition of diatom detritus the planktonic phase must have contained many previously particle-associated community members. Previous research has indicated that there were no statistically significant differences between the planktonic and particle-associated *Flavobacteria* communities in Southern Ocean samples [20]. It was surmised that this was due to bacterial cells dispersing from particles as the particles decompose and sink, a phenomenon originally proposed by Azam [46]. Thus, the planktonic population at T0 in the mesocosm likely included species poised to take advantage of the resupply of particulate matter. The different responses observed between the three mesocosms seems to reflect inherent differences in the pre-existing communities. Clearly, dynamic Flavobacteria colonization in microcosm S1 was favored and the initial high abundance of *Flavobacteria* (Fig. 3) may have been a factor. *Flavobacteria* abundance on the particulate matter in S1 was up to 4-times higher than the other samples (Fig. 3). The mesocosm incubation temperature of 2 °C was low enough to be sub-optimal for the growth of all organisms present [47] which suggests organisms at a high initial biomass could have had a potential advantage in colonizing the detrital material. Colonization would also be eventually influenced by many other factors including predation by nanoflagellates (that may have passed through the 0.8 μm filter), substrate acquisition strategies, cell to cell and cell to surface interactions, biochemical and physiological constraints (e.g., temperature and viscosity) and antagonistic and beneficial influences within the chemical environment [48–51]. Competition from Proteobacteria in S1 may have also been more muted, as α-Proteobacteria and γ-Proteobacteria were initially less predominant. On the other hand Proteobacteria in mesocosms S2 and S3 seem to rapidly predominate potentially hindering their colonization of the detritus (Fig. 2); only the most responsive and competitive *Flavobacteria* appeared to be able to become a dominant part of the new detrital community. This is consistent with the concept that there is the tendency for the most productive species to be the most common, keeping other taxa at relatively lower proportions [52]. Interestingly, the sudden appearance of DGGE band S205 late in the incubation of mesocosm S2 also suggests that conditions in the mesocosms may actually change to favor certain species present at initially undetectable levels and also suggests that community shifts were still occurring in the latter stages of the experiment. It is unclear whether S205's presence had anything to do with the sudden acceleration of silica released in the S1 mesocosm between days 25 and 30.

It was observed that the community structures stabilizing at the end of the mesocosm experiment seemed to some extent reflects the geography of the seawater sampling sites. This was most evident for mesocosm S3, which was dominated by *Polaribacter* phylotypes. The incidence of genus *Polaribacter* in the mesocosms from waters collected at lower latitudes (PFZ and ice edge zone of the AZ) was less evident, with only one DGGE band detected in mesocosm S2 and none at all in the relatively more diverse community of mesocosm S1. From molecular surveys there is good evidence that *Polarib-*

acter is "endemic" to the waters of the AZ ice pack. *Polaribacter* phylotypes were detected in most AZ samples but undetectable in samples collected north of the Polar Front [20]. *Polaribacter* is also strongly associated with sea-ice algal assemblages and occurs in Arctic Ocean waters and sea-ice [18,19]. The genus was easy to isolate from AZ samples (Section 3.6, Fig. 5) and thus the overall data suggests this genus likely plays a significant role in secondary production in high latitude seawater and sea-ice. The temperature differential between the experiments (performed at 2 °C) and the original temperature of the samples (from −1 to 2 °C) may have influenced the community structure obtained in the mesocosms. nMDS and ANOSIM analysis of DGGE band patterns of PFZ and AZ surface water samples, which ranged in temperature from −1.4 to 5.6 °C, however demonstrate the Flavobacteria communities present were relatively similar [20].

Interestingly, several of the DGGE band phylotypes detected in the three experiments grouped closely (sequence similarity >98%) with Southern Ocean seawater isolates (Table 1, Fig. 5). This suggests that these colonizing species could play a significant role in the degradation of particulate matter and in silica cycling in the Southern Ocean. However, many clades identified in a molecular survey containing uncultured *Flavobacteria*, namely clades I–VIII and agg58 cluster members, were not detected even though many of these were also detected in particulate fractions of the AZ and other zones of the Southern Ocean [20]. It is notable that many species manifesting DGGE bands present at T0 in the mesocosms, especially in mesocosms S2 and S3, did not colonize the detritus. Though these bands were not sequenced it is possible that these bands represented phylotypes belonging to uncultured clades as several were found in all or most Southern Ocean samples [20], in both the particulate and planktonic fractions, especially clades II, III, V and VII (Fig. 5). This suggests that many *Flavobacteria* present in the original seawaters sample did not colonize the detritus under the conditions provided. Several reasons can be advanced to explain this, all of which will require new experiments to be performed to assess this problem. The uncultured species could be comparatively slow growing [53]. As a result other fast growing bacteria including various *Flavobacteria* could exclude the uncultured species because they rapidly colonies surfaces on the detrital particles. Alternatively, various rapidly colonizing bacteria may produce substances that can inhibit other bacteria in order to maximize their competitiveness. This phenomenon has been shown in many instances in the marine ecosystem and various studies show that this trait is particularly common amongst the *Proteobacteria* [50], which became dominant on the detrital particles in mesocosms S2 and S3. Required growth factors may not have been available in the detritus and the surrounding seawater. It is possible the colonization of some taxa may only occur after fast growing bacteria exhaust common nutrients and exude growth promoting secondary metabolites [54]. Finally, a broader explanation is hypothesized. Uncultured *Flavobacteria* and other not easily cultured bacterial types may exist in stable arrangements with various different phytoplankton species under a state of pronounced nutrient limitation and that cell populations have slowly accumulated over time to sustainable levels. In other words they could represent classic *K*-type strategists. Since the uncultured populations would likely have initial abundance advantage compared to the smaller numbers of the fast growing and opportunistic (*r*-strategist) species they can take effective advantage of the transient appearance of nutrients. Indeed, this exemplifies the concept that the mesocosm experiment was affected by a treatment effect in which nutrients were released in such high concentrations that *r*-strategist populations could become quickly dominate while *K*-strategist populations were unable to make any inroads during the short experiment. Longer-term mesocosm experiments performed with diffusion chambers, allowing exchange of seawater and preventing rapid build-up of dissolved organic compounds may allow colonization by various uncultured *Flavobacteria* clade members.

5. Conclusions

In this mesocosm study, we observed *Flavobacteria* as well as *Proteobacteria* rapidly colonized diatom detritus and that community members involved in the colonization were initially abundant in the initial seawater samples. Substantial differences in sample communities that pre-existed before the experiments were preserved, which may suggest that initial abundance could be a significant factor in bacterial colonization of detritus though other factors physical, biological and chemical likely play some part, resulting in different responses between different samples. The lack of certain groups previously detected in Southern Ocean water, however also demonstrates that responses by *Flavobacteria* to nutrient availability varies considerably. Riemann et al. [8] proposed specialist bacteria performed detritus colonization. Results here seem to partially support this, especially in the case of the *Tenacibaculum* species, however the shear diversity of *Flavobacteria* involved in the detritus colonization suggests that a large fraction of the *Flavobacteria* community are capable of engaging in colonization and that colonization is perhaps not determined substantially by the specific nature of the particulate matter. Determining the specific parameters defining colonization ability and nutrient responses between different bacterial taxa would be important for better understanding of secondary production processes.

References

[1] Karl, D.M. (1992) Microbial processes in the Southern OceansAntarctic Microbiology, pp. 1–63. Wiley-Liss, New York.

[2] Kopczynska, E.E., Dehairs, F., Elskens, M. and Wright, S. (2001) Phytoplankton and microzooplankton variability between the Subtropical and Polar Fronts south of Australia: Thriving under regenerative and new production in late summer. J. Geophys. Res.-Oceans 106, 31597–31609.

[3] Cole, J.J, Findlay, S. and Pace, M.L. (1988) Bacterial production in fresh and saltwater ecosystems – a cross-system overview. Mar. Ecol. Prog. Ser. 43, 1–10.

[4] Paerl, H.W. and Steppe, T.F. (2003) Scaling up: the next challenge in environmental microbiology. Environ. Microbiol. 5, 1025–1038.

[5] Bidle, K.D. and Azam, F. (1999) Accelerated dissolution of diatom silica by marine bacterial assemblages. Nature 397, 508–512.

[6] Bidle, K.D. and Azam, F. (2001) Bacterial control of silicon regeneration from diatom detritus: significance of bacterial ectohydrolases and species identity. Limnol. Oceanogr. 46, 1606–1623.

[7] Bidle, K.D., Brzezinski, M.A., Long, R.A., Jones, J.L. and Azam, F. (2003) Diminished efficiency in the oceanic silica pump caused by bacteria-mediated silica dissolution. Limnol. Oceanogr. 48, 1855–1868.

[8] Riemann, L., Steward, G.F. and Azam, F. (2000) Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. Appl. Environ. Microbiol. 66, 578–587.

[9] Prieto, L., Ruiz, J., Echevarria, F., Garcia, C.M., Bartual, A., Galvez, J.A., Corzo, A. and Macias, D. (2002) Scales and processes in the aggregation of diatom blooms: high time resolution and wide size range records in a mesocosm study. Deep-Sea Res. I. 49, 1233–1253.

[10] Lebaron, P., Servais, P., Troussellier, M., Courties, C., Vives-Rego, J., Muyzer, G., Bernard, L., Guindulain, T., Schafer, H. and Stackebrandt, E. (1999) Changes in bacterial community structure in seawater mesocosms differing in their nutrient status. Aquat. Microb. Ecol. 19, 255–267.

[11] Pinhassi, J., Azam, F., Hemphala, J., Long, R.A., Martinez, J., Zweifel, U.L. and Hagstrom, Å. (1999) Coupling between bacterioplankton species composition, population dynamics, and organic matter degradation. Aquat. Microb. Ecol. 17, 13–26.

[12] Schafer, H., Bernard, L., Courties, C., Lebaron, P., Servais, P., Pukall, R., Stackebrandt, E., Troussellier, M., Guindulain, T., Vives-Rego, J. and Muyzer, G. (2001) Microbial community dynamics in Mediterranean nutrient-enriched seawater mesocosms: changes in the genetic diversity of bacterial populations. FEMS Microbiol. Ecol. 34, 243–253.

[13] Joint, I., Henriksen, P., Fonnes, G.A., Bourne, D., Thingstad, T.F. and Riemann, B. (2002) Competition for inorganic nutrients between phytoplankton and bacterioplankton in nutrient manipulated mesocosms. Aquat. Microb. Ecol. 29, 145–159.

[14] Grossart, H.P., Riemann, L. and Azam, F. (2001) Bacterial motility in the sea and its ecological implications. Aquat. Microb. Ecol. 25, 247–258.

[15] Heukelekian, H. and Heller, A. (1940) Relation between food concentration and surface for bacterial growth. J. Bacteriol. 40, 547–558.

[16] Glöckner, F.O., Fuchs, B.M. and Amann, R. (1999) Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence in situ hybridization. Appl. Environ. Microbiol. 65, 3721–3726.

[17] Simon, M., Glöckner, F.O. and Amann, R. (1999) Different community structure and temperature optima of heterotrophic picoplankton in various regions of the Southern Ocean. Aquat. Microb. Ecol. 18, 275–284.

[18] Brown, M.V. and Bowman, J.P. (2001) A molecular phylogenetic survey of sea-ice microbial communities (SIMCO). FEMS Microbiol. Ecol. 35, 267–275.

[19] Brinkmeyer, R., Knittel, K., Jurgens, J., Weyland, H., Amann, R. and Helmke, E. (2003) Diversity and structure of bacterial communities in Arctic versus Antarctic Pack Ice. Appl. Environ. Microbiol. 69, 6610–6619.

[20] Abell, G.C.J. and Bowman, J.P. (2005) Ecological and biogeographic relationships of class *Flavobacteria* in the Southern Ocean. FEMS Microbiol. Ecol. 51, 265–277.

[21] Rath, J., Wo, K.Y., Herndl, G.J. and DeLong, E.F. (1998) High phylogenetic diversity in a marine-snow-associated bacterial assemblage. Aquat. Microb. Ecol. 14, 261–269.

[22] O'Sullivan, L.A., Fuller, K.E., Thomas, E.M., Turley, C.M., Fry, J.C. and Weightman, A.J. (2004) Distribution and culturability of the uncultivated 'AGG58 cluster' of the *Bacteroidetes* phylum in aquatic environments. FEMS Microbiol. Ecol. 47, 359–370.

[23] Fandino, L.B., Riemann, L., Steward, G.F., Long, R.A. and Azam, F. (2001) Variations in bacterial community structure during a dinoflagellate bloom analysed by DGGE and 16S rDNA sequencing. Aquat. Microb. Ecol. 23, 119–130.

[24] Pinhassi, J., Sala, M.M., Havskum, H., Fransesc, P., Guadayol, O., Malits, A. and Marrasé, C. (2004) Changes in bacterioplankton composition under different phytoplankton regimes. Appl. Environ. Microbiol. 70, 6753–6766.

[25] Cottrell, M.T. and Kirchman, D.L. (2000) Natural assemblages of marine Proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter. Appl. Environ. Microbiol. 66, 1692–1697.

[26] Janse, I., Zwart, G., van de Maarel, M.J.E.C. and Gottschal, J.C. (2001) Composition of the bacterial community degrading *Phaeocystis* mucopolysaccharides in enrichment cultures. Aquat. Microb. Ecol. 22, 119–133.

[27] Covert, J.S. and Moran, M.A. (2001) Molecular characterization of estuarine bacterial communities that use high- and low-molecular weight fractions of dissolved organic carbon Aquat. Microb. Ecol. 25, 127–139.

[28] Bowman, J.P., Mancuso Nichols, C.A. and Gibson, J.A.E. (2003) *Algoriphagus ratkowskyi* gen. nov., sp. nov., *Brumimicrobium glaciale* gen. nov., sp. nov., *Cryomorpha ignava* gen. nov., sp. nov. and *Crocinitomix catalasitica* gen. nov., sp. nov., novel flavobacteria isolated from various polar habitats. Int. J. Syst. Evol. Microbiol. 53, 1343–1355.

[29] Kirchman, D.L., Yu, L. and Cottrell, M.T. (2003) Diversity and abundance of uncultured *Cytophaga*-like bacteria in the Delaware Estuary. Appl. Environ. Microbiol. 69, 6587–6596.

[30] DeLong, E.F., Franks, D.G. and Alldredge, A.L. (1993) Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. Limnol. Oceanogr. 38, 924–934.

[31] Graßhoff, K. (1964) On the determination of silica in seawater. Deep Sea Res. 11, 597–604.

[32] Weller, R., Glöckner, F.O. and Amann, R. (2000) 16S rRNA-targeted oligonucleotide probes for the in situ detection of members of the phylum *Cytophaga-Flavobacterium-Bacteroides*. Syst. Appl. Microbiol. 23, 107–114.

[33] Fuhrman, J.A., Comeau, D.E., Hagstrom, Å. and Chan, A.M. (1988) Extraction from natural planktonic microorganisms of DNA suitable for molecular biological studies. Appl. Environ. Microbiol. 54, 1426–1429.

[34] Lane, D.J. (1991) 16S/23S rRNA sequencing In: Nucleic Acid Techniques in Bacterial Systematics (Stackebrandt, E. and Goodfellow, M., Eds.), pp. 115–175. John Wiley & Sons, New York.

[35] Ferris, M.J., Muyzer, G. and Ward, D.M. (1996) Denaturing gradient gel electrophoresis profiles of 16S rRNA-defined popu-

lations inhabiting a hot spring microbial mat community. Appl. Environ. Microbiol. 62, 340–346.
[36] Powell, S.M., Bowman, J.P., Snape, I. and Stark, J.S. (2003) Microbial community variation in pristine and polluted nearshore Antarctic sediments. FEMS Microbiol. Ecol. 45, 135–145.
[37] Rappé, M.S., Connon, S.A., Vergin, K.L. and Giovannoni, S.J. (2002) Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. Nature 418, 630–633.
[38] Marmur, J. and Doty, P. (1962) Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. Mol. Biol. 5, 109–118.
[39] Hall, T.A. (1999) A user-friendly biological sequence alignment editor for Windows 95/98/NT. Nucl. Acids Symp. Ser. 41, 95–98.
[40] Felsenstein, J. PHYLIP, phylogenetic inference package. 1993. Available from: http://evolution.genetics._washington.edu/phylip.html.
[41] Bowman, J.P., Nichols, D.S. Novel members of the family *Flavobacteriaceae* from Antarctic maritime habitats including *Subsaximicrobium* gen. nov, *Subsaxibacter* gen. nov., *Lacinutrix* gen. nov., and new species of the genera *Biziolla*, *Gelidibacter* and *Gillisia*. Int. J. Syst. Evol. Microbiol., DOI: 10.1099/ijs.0.63527-0, in press.
[42] Riemann, L., Steward, G.F., Fandino, L.B., Campbell, L., Landry, M.R. and Azam, F. (1999) Bacterial community composition during two consecutive NE monsoon periods in the Arabian Sea studied by denaturing gradient gel electrophoresis (DGGE) of rRNA genes. Deep Sea Res. II 46, 1791–1811.
[43] Casamayor, E.O., Schafer, H., Baneras, L., Pedros-Alio, C. and Muyzer, G. (2000) Identification of and spatio-temporal differences between microbial assemblages from two neighboring sulfurous lakes: Comparison by microscopy and denaturing gradient gel electrophoresis. Appl. Environ. Microbiol. 66, 499–508.
[44] Kan, J., Wang, K., Chen, F. Are 5000 bacterial cells per ml the detection threshold for PCR-DGGE annalysis? in: 10th International Symposium on Microbial Ecology, Cancun, Mexico, August 22–27, 2004, p. 266.
[45] Gleitz, M., Grossmann, S., Scharek, R. and Smetacek, V. (1996) Ecology of diatom and bacterial assemblages in water associated with melting summer sea ice in the Weddell Sea, Antarctica. Antarctic Sci. 8, 135–146.
[46] Azam, F. (1998) Microbial control of oceanic carbon flux: The plot thickens. Science 280, 694–696.
[47] Ratkowsky, D.A., Olley, J. and Ross, T. (2005) Unifying temperature effects on the growth rate of bacteria and the stability of globular proteins. J. Theoret. Biol. 233, 351–362.
[48] Kiorboe, T. and Jackson, G.A. (2001) Marine snow, organic solute plumes, and optimal chemosensory behavior of bacteria. Limnol. Oceanogr. 46, 1309–1318.
[49] Grossart, H.P., Kiorboe, T., Tang, K. and Ploug, H. (2003) Bacterial colonization of particles: Growth and interactions. Appl. Environ. Microbiol. 69 (6), 3500–3509.
[50] Meiners, K., Brinkmeyer, R., Granskog, M.A. and Lindfors, A. (2004) Abundance, size distribution and bacterial colonization of exopolymer particles in Antarctic sea ice (Bellingshausen Sea). Aquat. Microbiol. Ecol. 35, 283–296.
[51] Long, R.A. and Azam, F. (2001) Antagonistic interactions among marine pelagic bacteria. Appl. Environ. Microbiol. 67, 4975–4983.
[52] Chesson, P., Pacala, S.W. and Neuhauser, C. (2002) Environmental niches and ecosystem functioning In: The Functional Consequences of Biodiversity: Empirical Progress and Theoretical Extensions (Kinzig, A.P., Pacala, S.W. and Tilman, D., Eds.), pp. 213–245. Princeton University, Press, Princeton, NJ.
[53] Hahn, M.W., Stadler, P., Wu, Q.L. and Pockl, M. (2004) The filtration-acclimatization method for isolation of an important fraction of the not readily cultivable bacteria. J. Microbiol. Meth. 57, 379–390.
[54] Kaeberlein, T., Lewis, K. and Epstein, S.S. (2002) Isolating ''uncultivable'' microorganisms in pure culture in a simulated natural environment. Science 296, 1127–1129.