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Microbial diversity in a Pacific Ocean transect from the Arctic to Antarctic circles

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ABSTRACT: Microbial diversity in surface waters was examined along a ~15 400 km transect of the Pacific Ocean from 70° N to 68° S latitude between late August and early November 2003. Comparative microbial diversity was determined using terminal restriction fragment length polymorphism (T-RFLP) analysis of PCR amplified 16S and 18S rDNA restriction digested with *Cfo*I and *Msp*I. Bacterial numbers and total chlorophyll were greatest at higher latitudes in both hemispheres, with a smaller peak in equatorial waters. Flow cytometry analysis indicated a strong peak in *Prochlorococcus* from approximately 30° N to 30° S. Richness at each station was relatively low, with ~11 prokaryotic peaks per sample and ~12 eukaryotic peaks per community. Overall, prokaryotic populations appeared more diverse, with 181 total terminal restriction fragments (T-RFs) generated, while eukaryotic populations produced a total of 135 T-RFs. Prokaryotic and eukaryotic similarity dendrograms revealed 4 distinct cluster groups relating to regions sub-Arctic/Arctic, temperate, tropical and sub-Antarctic/Antarctic. T-RFLP patterns suggest that microbial communities may be influenced by ambient water temperature, with mid-latitudinal and equatorial communities more similar in composition to each other than to cold water communities. Global distribution of prokaryotic communities revealed an average inter-group similarity of ~52 %, while eukaryotic communities showed ~51 % similarity, implying that Pacific planktonic communities appear to be fairly homogenous in composition. Several T-RFs were ubiquitously distributed and contributed significantly to each cluster group, while several T-RFs were observed to be endemic to particular oceanic regions. Within-group similarities of >70 % were attributed to 12–14 T-RFs and 8–11 T-RFs in prokaryotic and eukaryotic profiles, respectively, suggesting that a small number of phylogenetic groups were responsible for each cluster group.

KEY WORDS: Microbial diversity · Pacific Ocean · T-RFLP · 16S rDNA · 18S rDNA · Biogeographical distribution

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INTRODUCTION

In order to understand planktonic diversity and the roles of microorganisms in global biogeochemical cycling, we must recognize the distribution patterns of planktonic phylotypes and their controlling environmental factors. The introduction of molecular technologies has revolutionized our view of the microbial world, resulting in a dramatic increase in the number of microbial diversity studies. Before the 1990s, investigations of microbial diversity were driven primarily by culture-based methodologies until Giovannoni et al. (1990) reported the results of a phylogenetically analyzed clone library from the Sargasso Sea. Using 16S rRNA genes, the novel clade SAR11 and the oxygenic phototrophs (cyanobacteria, *Synechococcus*, *Prochlorophytes*) were revealed as dominant members of the resident bacterioplankton community (Giovannoni et

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al. 1990). Continual advancement and application of molecular-based technologies has revealed the presence of marine microorganisms that were not detectable by earlier culture based methods (Giovannoni et al. 1990, Fuhrman et al. 1993, DeLong 1998). Molecular methods have revolutionized our understanding of marine microbial communities in terms of the composition, phylogeny, function and physiology within the environment, and they provide further insight into the overall biogeochemical capabilities of microbial communities. These studies contribute greatly to the ever-expanding database of known microbial DNA sequences (Benson et al. 2004), thus aiding further studies of microbial diversity. While commonly used SSU rRNA (small subunit ribosomal RNA) gene 'markers' have limitations, they are an effective means for providing an initial phylogenetic framework from which physiological and ecological impacts can be determined and for garnering an overall view of microbial diversity from single or multiple habitats.

Diversity studies of marine bacterioplankton have revealed that ~80 % of marine bacteria fall within only 9 phylogenetic groups which form clusters of related genes (not single lineages) and appear to be globally distributed (Giovanonni & Rappé 2000). Of these 9 groups, only 2 have had representatives that have been successfully cultured, indicating the substantial need for molecular-based approaches, especially when examining large-scale distribution patterns of marine bacterioplankton.

In contrast to bacterial phylogenetics using SSU rRNA genes, molecular-based protistan diversity studies have lagged behind due to well-established morphology-based taxonomy. Many protistan groups are of sufficient size to allow for identification based on morphological characteristics alone, although there are concerns about the reliability of morphology for classification purposes (Darling et al. 2000, Norris & de Vargas 2000). Molecular-based methods enable a more thorough characterization of protistan diversity over a wider range of sampling locations in comparison to culture-dependent techniques and microscopy. These morphology and culture-independent approaches are revealing higher levels of protistan diversity in a variety of ecosystems (Diez et al. 2001, Moon-van der Staay et al. 2001, Gast et al. 2004, Countway et al. 2005).

Most recent microbial diversity studies have been PCR based. Methods such as denaturing gradient gel electrophoresis (DGGE), cloning, sequencing and terminal restriction fragment length polymorphism (T-RFLP) allow for the identification of individual species (operational taxonomic units [OTU]) in a heterogenous community based on phylogenetic markers such as 16S rDNA and 18S rDNA (Ward et al. 1990, Diez et al. 2001). T-RFLP is a technique wherein a gene of interest (e.g. 16S or 18S rRNA) is amplified from a heterogenous community using a fluorescently labeled primer. Following digestion with restriction endonucleases, variably sized terminal fragments can be differentiated with high resolution (±1 base) through use of an automated DNA sequencer allowing researchers to generate 'fingerprints' of complex microbial communities (Liu et al. 1997). This readily applied technique has been shown to be effective in the comparison of microbial assemblages in various marine and terrestrial environments (Urakawa et al. 2001, Mummey & Stahl 2003, Gomez et al. 2004). Both prokaryotic and eukaryotic diversity have been documented in specific oceanic regions, but not on global scales. Herein we present a T-RFLP-based broad characterization of both the prokaryotic and eukaryotic components of the microbial communities in surface waters collected from the central Pacific Ocean from the Arctic to Antarctic circles during a ship of opportunity cruise: Production Observations Through Another Trans-latitudinal Oceanic Expedition (POTATOE).

MATERIALS AND METHODS

Sample collection. Samples were collected at 40 stations along a ~15 400 km transect of the Pacific Ocean on the RVIB 'Nathaniel B. Palmer' from 70° N to 68° S latitude between late August and early November 2003 (Fig. 1). Daily surface water samples were collected before sunrise at ~370 km intervals via bucket casting or Go-Flo bottles (General Oceanics) attached to a CTD rosette. Continuous measurements of surface salinity and temperature along the entire north–south transect were collected by a SeaBird SBE21 underway CTD. Samples for phylogenetic analysis (1 l) were filtered onto 0.2 µm 47 mm Supor 200 filters (Pall), folded loosely, placed into 2 ml microcentrifuge tubes and stored at –80°C until further processing off ship.

Bacterial production. Bacterial production was measured via the incorporation of ^{3}H-leucine (Smith & Azam 1992). Sample water (20 ml) was amended with ^{3}H-leucine (final concentration 10 nM). Aliquots of 5 ml were placed into 4 WhirlPak bags, one of which contained 0.5 ml of 100 % TCA as a killed control, and incubated for 4 h in the dark in flowing seawater tables. Incubations were terminated by the addition of TCA to a final concentration of 5 %. Samples were processed using the microfuge method of Smith & Azam (1992).

Chlorophyll *a* concentration and bacterial cell abundance. Chlorophyll samples were collected in duplicate 500 ml volumes on GF/F filters (Millipore),

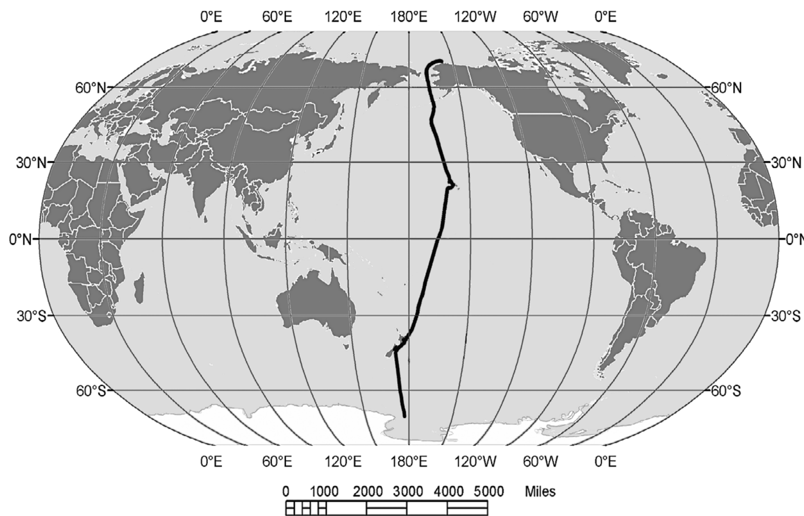

Fig. 1. Transect of sampling sites during the POTATOE cruise, August to November 2003

extracted in 90% acetone and quantified fluorometrically (Welschmeyer 1994). Bacterial cell concentrations were measured by epifluorescence microscopy of filtered-formalin preserved (2% final concentration, stored at 4°C) DAPI-stained samples (Porter & Feig 1980).

Nutrient analysis. Samples for inorganic nutrients were collected in 10% HCl and sample rinsed polyethylene bottles and frozen (–80°C) immediately. Nutrient concentrations were measured post-cruise by automated methods (US EEPA 1983).

Flow cytometry analysis of phytoplankton. Samples (3 ml) for flow cytometric analysis of phytoplankton were fixed with 2% formaldehyde (final concentration) in cryotubes held at room temperature for 10 to 15 min and then stored at –80°C until analysis. The samples were thawed at room temperature and analyzed on a flow cytometer (FACSCalibur, Becton Dickinson) equipped with a 488 nm, 15 mW Argon laser. Samples were run at ~60 µl min^{-1} for 5 min. The data were collected, stored and displayed on 4 decade scales using CELLQuest software (Becton Dickinson). Orange fluorescence (from phycoerythrin) was collected through a 585/42 nm filter and red fluorescence (from chlorophyll) through a 650 nm LP filter. According to their flow cytometric signatures, 4 phytoplanktonic groups were distinguished. *Synechococcus* cells were easily recognized by their orange fluorescence. *Prochlorococcus* had lower red fluorescence and right angle-light side scatter (SSC) signals than *Synechococcus*. Eukaryotic phytoplankton had higher fluorescence and SSC signals than *Synechococcus* and were divided into 2 size-classes (small and large cells) tentatively identified as pico- and nanophytoplankton cells. The red fluorescence was used as a threshold parameter. The settings (voltage of SSC and fluorescence photomultiplier tubes) were fixed and cell characteristics were normalized to fluorescent beads signals (1.0 µm; Polysciences).

Nucleic acid extraction. Samples were extracted for community analysis using the autoclave DNA extraction method of Simmon et al. (2004). This consisted of crushing a frozen filter inside the microfuge tube, adding 0.5 ml 1× TE (10 mM Tris-HCl, pH 7.5; 1 mM EDTA), vortexing to loosen cells bound to filter pieces, and autoclaving in a pre-heated chamber (AMSCO 3024-S isothermal sterilizer) under the following conditions: 10 s purge, sterilization at 121°C for 1 min and fast exhaust. The lysate was removed and placed onto a Centricon YM-100 spin column (Millipore) to concentrate and purify the DNA. The column was spun in a refrigerated centrifuge at 2630 rpm (1500 × *g*) for 20 min. The column was subsequently washed twice with 0.75 ml 1× TE and spun for 30 min. Columns were inverted and purified DNA was collected.

PCR amplification of 16S rDNA. Purified DNA samples were quantified using a GeneQuant *pro* (Amersham Biosciences) spectrophotometer. Ten to 100 ng of DNA were used per 50 µl PCR reaction. Genes encoding for 16S rRNA were amplified for T-RFLP analysis with the universal eubacterial fluorescently labeled (6-carboxyfluorescein [6-FAM]) forward primer 27F-(5′-6-FAM-AGAGTTTGATC(A/C)TGGCTCAG-3′) and the reverse primer 1492R (5′-GG(C/T)TACCTTGTTACGACTT-3′), producing an amplicon of ~1500 bp (Lane 1991).

PCR was performed in triplicate for each sample with the following final reagent concentrations in 50 µl: 0.2 µM each primer, 0.2 mM each TaKaRa *Ex Taq* dNTPs (Takara Bio), 1× *Ex Taq* buffer (2 mM Mg^{2+}), 0.2 mg ml^{-1} BSA (10 mg ml^{-1}; Promega), 1.25 U $µl^{-1}$ *Ex Taq* polymerase, 10 to 100 ng DNA template and brought to a final volume of 50 µl with sterile diethylpyrocarbonate (DEPC)-treated water (Fisher Scientific). PCR amplification was performed under the following thermal conditions: initial denaturation at 95°C for 1.5 min, 30 cycles at 95°C for 30 s, 50°C for 1 min, 72°C for 1.5 min, and a final extension at 72°C for 7 min with a hold at 4°C.

PCR products used for T-RFLP analysis were purified from a 0.8% agarose gel run in 1× TAE buffer with 0.05 µg ml^{-1} ethidium bromide. PCR products were isolated from the gel and purified with the QIAprep spin kit (QIAGEN) according to the manufacturer's instructions. Of each purified sample, 10 µl were then digested with the restriction endonucleases *Cfo*I and

*Msp*I (New England Biolabs) in separate 40 µl reactions at 37°C for 4 h. Digested DNA was precipitated for 1 h at –20°C with 0.1 volumes 3 M NaOAc (pH 5.2) and 2.5× volume of cold 95% ethanol (molecular biology grade). DNA was pelleted at 13 000 rpm (9500 × *g*) at 4°C for 1 h. The DNA pellet was desalted with 70% ethanol, air-dried and re-suspended in 20 µl sterile DEPC-treated water.

PCR amplification of 18S rDNA. Purified and quantified DNA samples were amplified for analysis by T-RFLP of the 18S rRNA gene using the fluorescently labeled forward primer (6-FAM)-EUK-A (5'-6-FAM-AACCTGGTTGATCCTGCCAGT-3') and the reverse primer EUK-570R (5'-GCTATTGGAGCTGGAATTAC-3'), which yielded an amplicon of ~600 bp (Countway et al. 2005). PCR was performed in triplicate for each sample, with the following final reagent concentrations in 50 µl: 0.5 µM each primer, 0.2 mM each TaKaRa *Ex Taq* dNTPs, 1× *Ex Taq* buffer (2 mM Mg^{2+}), 0.2 mg ml^{-1} BSA (10 mg ml^{-1}; Promega), 1.25 U $µl^{-1}$ *Ex Taq* polymerase, 10 to 100 ng template DNA and brought to a final volume of 50 µl with sterile DEPC-treated water.

PCR amplification was performed under the following thermal conditions: initial denaturation at 95°C for 2 min, 35 cycles at 95°C for 30 s, 55°C for 30 s, 72°C for 1 min, and a final extension at 72°C for 5 min with a hold at 4°C. Triplicate PCR products were pooled then cleaned and concentrated using the UltraClean PCR Clean-up Kit (Mo Bio Labs) according to the manufacturer's protocol. Concentrated PCR products (~1000 ng) were digested with 5 U mung bean nuclease (New England Biolabs) to eliminate the effect of pseudo-terminal restriction fragments (T-RFs) on the T-RFLP analysis (Egert & Frederich 2003). Mung bean digested PCR products were again purified and concentrated using the UltraClean PCR Clean-up Kit. Concentrated PCR products (50 µl) were gel purified from a 0.8% agarose gel run in 1× TAE buffer with 0.05 µg ml^{-1} ethidium bromide using the UltraClean 15 DNA Purification Kit (Mo Bio Labs; Countway et al. 2005). Of each sample, 5 µl were digested in a total volume of 20 µl with the restriction endonucleases *Cfo*I and *Msp*I in separate reactions at 37°C for 4 h. Digested DNA was precipitated as described above for 16S rDNA samples and re-suspended in 10 µl sterile DEPC-treated water.

T-RFLP analysis. Digested DNA (0.25 to 3 µl) was combined with 0.25 to 0.75 µl of DNA standard (MapMarker 1000, Bioventures) and denatured after the addition of 2 volumes of deionized formamide at 95°C for 5 min. Fragments were separated on an automated DNA sequencer (3100 Avant genetic analyzer, Applied Biosystems) in GeneScan mode (15 kV, 60°C for 40 min) using a 36 cm capillary and performance optimized polymer 4 (POP 4, Applied Biosystems). Fluorescence intensities of major peaks from community profiles were adjusted by increasing or decreasing template volumes and/or injection times to fall within 6500 to 8000 fluorescence units to ensure the comparability of profiles. The sizes of the 5'-T-RFs and corresponding fluorescence intensities (peak areas) were calculated using DAx® software (Data Acquisition and Analysis v7.0). A 150 fluorescence unit threshold value was used to separate true signals from background noise.

The richness of each community was estimated from the number of unique T-RFs in each restriction. In order to compare T-RFLP profiles, T-RFs from each community were binned according to fragment length (±1 bp). Relative abundance was estimated for each T-RF by dividing the peak area by the total peak area and multiplying by 100.

Resultant richness and abundance data were analyzed for comparison of communities. Similarity dendrograms were constructed using Primer5© software (Primer-E) applying the Bray-Curtis coefficient onT-RF relative peak abundances (log transformed) with the complete linkage similarity coefficient. SIMPER analysis (Primer5© software) of microbial community T-RFLP profiles was used to compare similarities within assemblages, dissimilarities between assemblages and to identify individual T-RFs responsible for clustered assemblages.

RESULTS

Surface water temperature, chemistry, chlorophyll concentrations and bacterial abundance

Surface water temperatures were ~10°C in the northern Pacific, peaking between 35° N and 22° S to 27°C and decreasing to –0.3°C in the southern part of the ocean (Fig. 2A). A peak in nitrate concentrations was observed in the sub-Arctic/Arctic Pacific HNLC (High Nutrient Low Chlorophyll) Zone, where iron limitation results in the accumulation of unutilized nitrate (Chisholm & Morel 1991). A peak was also seen at the Equatorial Upwelling and the New Zealand Continental Shelf, where the spring bloom was underway (Fig. 2A). Chlorophyll concentration was greatest at 54° N (5.09 µg l^{-1}), with average values in the northern Pacific of ~2.42 µg l^{-1}; a substantial decrease in concentration was observed in the mid- to lower latitudes (~0.51 µg l^{-1}), and maximum concentration of 4.29 µg l^{-1} was found in the southern waters at 40° S (Fig. 2B). Bacterial abundance and production rates also showed latitudinal patterns, exhibiting bacterial cell concentrations of ~2.70×10^8 cells l^{-1} in the northern Pacific through 30° N, a decline from 30° N through 30° S to ~1.50×10^8 cells l^{-1}, and average cell numbers

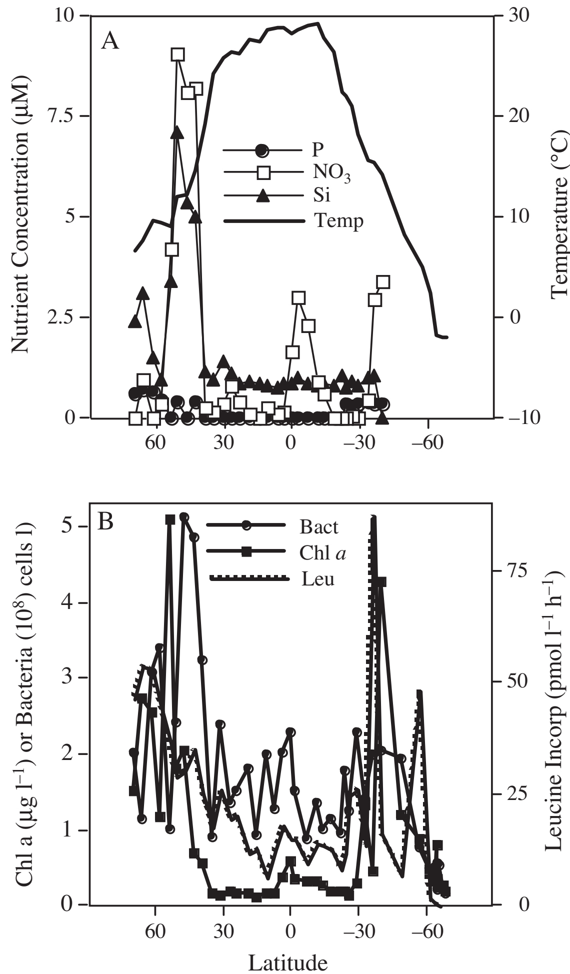

Fig. 2. Latitudinal trends in (A) surface orthophosphorus (P), nitrate (NO_3), silica (Si) and temperature; and (B) bacterial abundance (Bact), surface chlorophyll concentration (Chl *a*), and bacterial ^{3}H-leucine incorporation (Leu). Positive latitude values represent Northern Hemisphere locations and negative latitudes represent the Southern Hemisphere

of ~1.10×10^8 cells l^{-1} throughout southern Pacific waters (Fig. 2B). Bacterial production in the northern Pacific was ~39.40 pmol leucine (Leu) l^{-1} h^{-1}, decreased to ~14.50 pmol Leu l^{-1} h^{-1} in the mid- to lower latitudes and peaked at 36° S with 87.50 pmol Leu l^{-1} h^{-1} in the southern waters (Fig. 2B).

Flow cytometry

Flow cytometry analysis revealed the presence of *Prochlorococcus* between 35° N and 35° S (average abundance of ~4.38×10^7 cells l^{-1}) with a maximum value at the equator (1.10×10^8 cells l^{-1}; Fig. 3A). *Synechococcus* were present throughout the transect, with ~5.75×10^6 cells l^{-1} in northern Pacific waters, a slight increase in average abundance throughout the mid- to

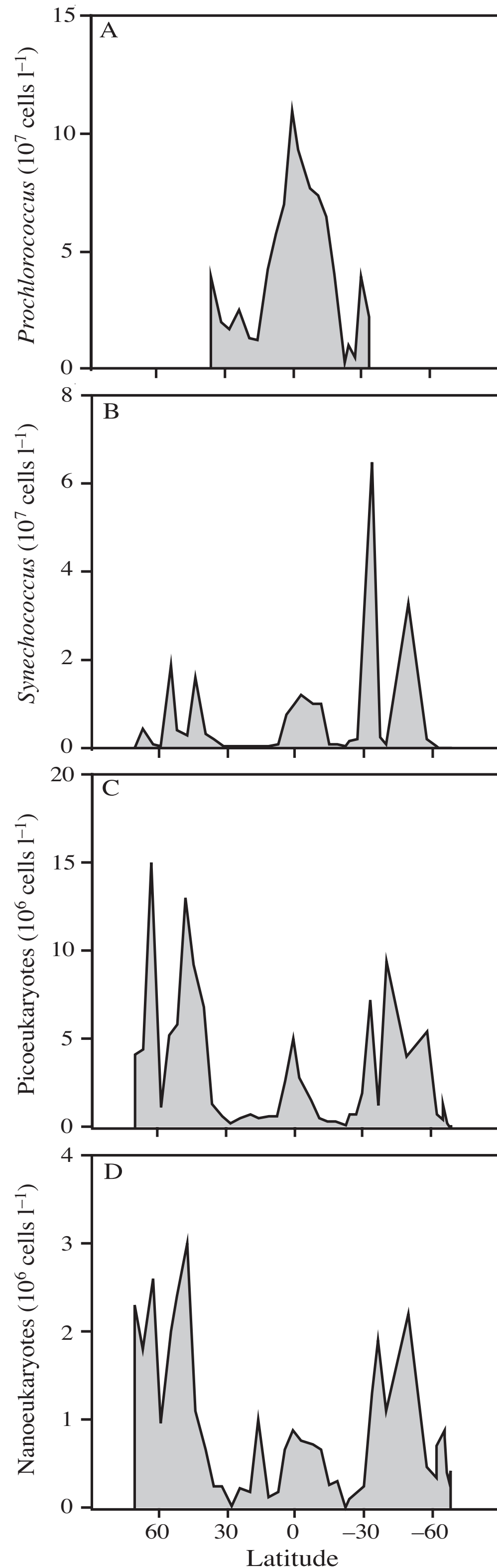

Fig. 3. Flow cytometric abundances of (A) *Prochlorococcus*, (B) *Synechococcus*, (C) picoeukaryotes and (D) nanoeukaryotes. Positive latitude values represent Northern Hemisphere locations and negative latitudes represent the Southern Hemisphere

lower latitudes (~7.60 × 10^6 cells l^{-1}), and a peak in abundance at 33° S (~6.50 × 10^7 cells l^{-1}; Fig. 3B). Photosynthetic picoeukaryote abundance followed latitudinal patterns as would be expected, with average abundance in the northern Pacific waters of ~7.18 × 10^6 cells l^{-1}, decreased abundance in the mid- to lower latitudes (~1.47 × 10^6 cells l^{-1}) and a slight elevation at 30° S of ~2.42 × 10^6 cells l^{-1} throughout the southern polar waters (Fig. 3C). Photosynthetic nanoeukaryote abundance also followed latitudinal trends with abundances in the northern Pacific and temperate/tropical regions of the Pacific of ~2.11 × 10^6 and ~3.38 × 10^5 cells l^{-1}, respectively and ~7.44 × 10^6 cells l^{-1} south from 30° (Fig. 3D).

Microbial community structure analysis: restriction endonuclease production of 5′-terminal restriction fragments

Prokaryotic and eukaryotic 5′ T-RFLP profiles were generated following separate digestions of PCR products from each location with the endonucleases *Cfo*I and *Msp*I. The number of T-RFs from each sample obtained through *Msp*I restriction of amplified 16S rDNA was greater than that determined with *Cfo*I generated fragments, with 8 to 38 and 4 to 20 T-RFs, respectively. PCR amplified 18S samples restricted with *Cfo*I and *Msp*I produced similar T-RF quantities of 5 to 23 and 6 to 25 T-RFs, respectively.

Ribotype richness

No latitudinal pattern of ribotype richness or community diversity between or amongst prokaryotic and eukaryotic profiles was found; however, the prokaryotic population appeared to have a higher ribotype richness with 181 total T-RFs produced, while eukaryotic populations produced 135 total T-RFs along the transect (data not shown). It is possible that this greater ribotype richness of the prokaryotic population is due to the lack of nuclease digestion prior to the T-RFLP analysis.

Cluster analysis of T-RFLP profiles

To assess similarity among prokaryotic and eukaryotic communities, T-RFLP patterns from each sampling location were implemented as a 'community fingerprint' based on the presence or absence of T-RFs and the relative amount of DNA for each T-RF. Communities analyzed through the use of hierarchical clustering of Bray-Curtis similarities indicated strong geographic integrity for 16S T-RFLP profiles but slightly less for the 18S profiles. Hierarchical analysis of 16S T-RFLP profiles indicated the presence of 4 distinct clusters—sub-Arctic/Arctic (Group I), tropical (Group II), temperate (Group III) and sub-Antarctic/Antarctic (Group IV)—apportioned according to oceanic regions, suggesting a relationship with temperature and its environmental correlates (Fig. 4). According to SIMPER analysis, prokaryotic cluster groups contained high within-group average similarities (~55 to 67%) and comparable inter-group similarity (average similarity 42 to 57%). Groups I and II exhibited 44% similarity, Groups I and III 51% similarity, Groups I and IV, II and IV, and III and IV exhibited 47, 42 and 44% similarity, respectively, while Groups II and III had the highest degree of similarity at 57%.

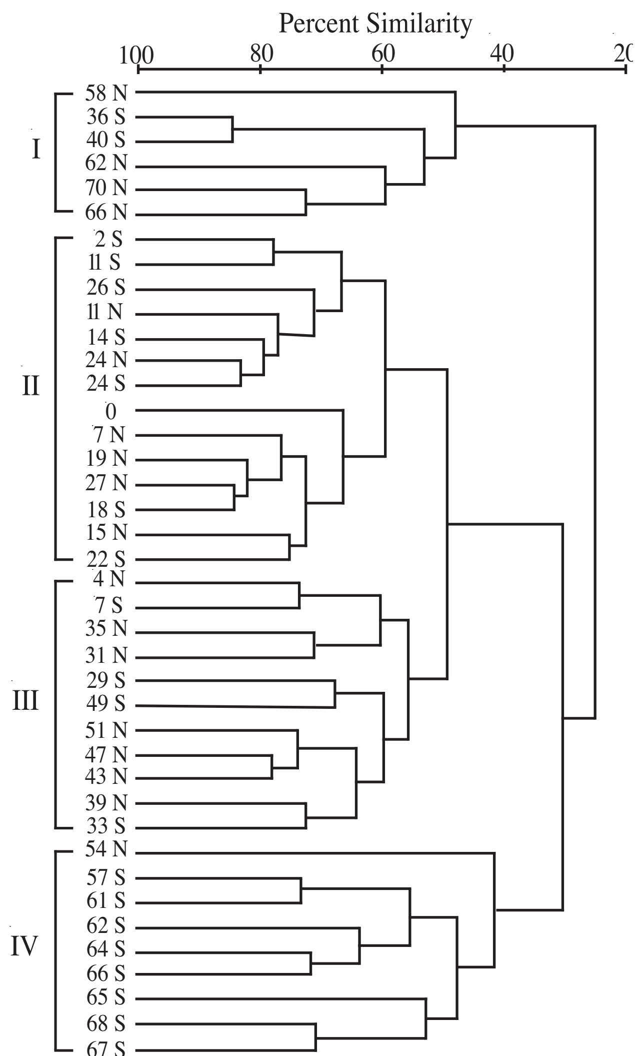

Fig. 4. 16S rDNA similarity dendrogram based on the presence/absence and relative abundances of *Cfo*I and *Msp*I restricted terminal restriction fragments (T-RFs)

Hierarchical analysis of 18S T-RFLP profiles produced 4 major clusters following the same oceanic groupings as 16S profiles — sub-Arctic/Arctic (Group IV), tropical (Group II), temperate (Group I) and sub-Antarctic/Antarctic (Group III) (Fig. 5), although the clusters were less congruent in their geographic constitution when compared to prokaryotic cluster assemblages. Eukaryotic assemblages had markedly high within-group average similarities (~50 to 62 %), with Groups III and IV showing the highest inter-group similarity (average similarity 53 %) compared to all other inter-group assessments. Groups I and II, Groups I and III and Groups I and IV exhibited 48, 47 and 49 % similarity, respectively, while Groups II and IV and Groups II and III exhibited 49 and 48 % similarity, respectively. Groups II and III had the highest degree of similarity at 53 %. Eukaryotic communities appeared to exhibit the same degree of within cluster similarity (51 %) over latitudinal gradients as the prokaryotic communities (52 %).

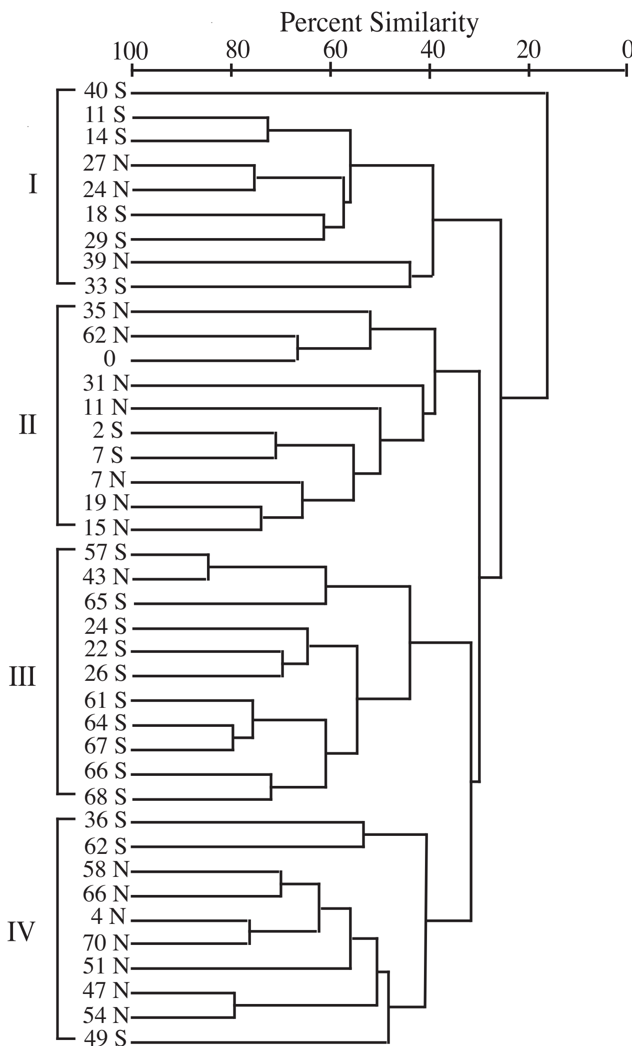

Fig. 5. 18S rDNA similarity dendrogram based on the presence/absence and relative abundances of *Cfo*I and *Msp*I restricted terminal restriction fragments (T-RFs)

SIMPER analysis of profiles was used to determine the magnitude and identity of the T-RFs responsible for similarities within and dissimilarities between each group. Bacterioplankton community analysis revealed that 12 to 14 T-RFs were responsible for >70 % of each of the 4 cluster's within-group similarities, while eukaryotic community analysis revealed 8 to 11 T-RFs as the contributors to >70 % of the 4 cluster's within-group similarities (Tables 1 & 2). These results, coupled with the order-level resolution of the T-RFLP analysis, indicate that a small number of phylogenetic groups contributed to each distinct assemblage. In addition to being major contributors to within-group similarities, several of these T-RFs were present in most or all of the 16S T-RFLP profiles, suggesting a cosmopolitan distribution. The *Cfo*I-derived 910 bp peak was consistently and substantially present in all profiles, contributing 26 to 92 % of the total community 16S rDNA and showing only lower relative abundance (10 to 35 % of the total) in northern Pacific waters. *Cfo*I-derived T-RFs 367, 564 and *Msp*I-derived T-RFs 145 and 489 were also present at all latitudes, but contributed only 3 and 6 % (367 and 564 bp) and 6 and 9 % (145 and 489 bp) to the overall community structure (Table 1). Phylogenetic group(s) comprising the 489 bp peak showed high abundance (32 %) in Group II, while the phylogenetic group(s) representing the 145 bp peak had an abundance of 25 % in Group IV (Table 1).

Complementary patterns were observed in the eukaryotic samples along the transect. Eukaryotic phylogenetic groups representing fragments at 172 and 433 bp (*Cfo*I-restricted) were present in all samples and contributed greatest to Group II (36 and 25 %) and least to Groups I and III (8 and 9 % for 172 bp and 8 % for 433 bp ; Table 2). *Msp*I-derived T-RFs 280 and 382 were also present in all samples, with T-RF 280 showing the greatest contribution to Group II (47 %) and the least to Groups I and III (8 and 9 %), and T-RF 382 showing an average contribution of 13 % to all groups (Table 2). *Cfo*I-restricted 419 and 426 bp T-RFs were present throughout the transect profiles, although their respective abundances were typically low (<3 %). Of the T-RFs commonly present within eukaryotic community profiles, the *Msp*I-derived 232, 240 bp T-RFs showed a considerable increase in abundance in sub-Antarctic/Antarctic T-RFLP profiles from sub-Arctic/Arctic and temperate/tropical profiles (1 to 4 %). As with prokaryotic profiles, several T-RFs varied in their presence and/or absence, with no apparent trends observed with respect to other T-RFs and/or latitude (data not shown).

Differences within prokaryotic clustering were primarily accounted for by T-RFs that were more

Table 1. Mean relative abundance (Abund., average values as percentage) and percent contribution (Contrib., to the within-group similarity) of 16S rDNA 5′-terminal restriction fragments (T-RFs) contributing to >70 % of within-group similarity. Average similarities: Group I = 58 %; Group II = 67 %; Group III = 60 %; Group IV = 55 %

Group I			Group II			Group III			Group IV		
T-RF (bp)	Abund.	Contrib.	T-RF (bp)	Abund.	Contrib.	T-RF (bp)	Abund.	Contrib.	T-RF (bp)	Abund.	Contrib.
910 *Cfo*I	31	8	910 *Cfo*I	64	11	910 *Cfo*I	74	12	910 *Cfo*I	67	12
90 *Cfo*I	24	8	439 *Msp*I	35	9	439 *Msp*I	25	9	145 *Msp*I	25	9
93 *Cfo*I	14	7	489 *Msp*I	32	9	145 *Msp*I	24	9	489 *Msp*I	10	7
145 *Msp*I	12	6	178 *Cfo*I	11	7	489 *Msp*I	17	8	367 *Cfo*I	5	6
55 *Cfo*I	12	6	668 *Cfo*I	16	6	367 *Cfo*I	6	6	439 *Msp*I	6	5
367 *Cfo*I	7	6	145 *Msp*I	8	6	137 *Msp*I	3	5	518 *Cfo*I	10	5
489 *Msp*I	11	5	367 *Cfo*I	4	5	542 *Msp*I	5	4	495 *Msp*I	11	4
96 *Cfo*I	4	5	450 *Msp*I	3	4	178 *Cfo*I	4	4	306 *Msp*I	6	4
542 *Msp*I	3	4	435 *Msp*I	2	4	90 *Cfo*I	2	4	93 *Cfo*I	4	4
137 *Msp*I	2	4	137 *Msp*I	2	4	88 *Msp*I	3	4	55 *Cfo*I	1	4
88 *Msp*I	6	4	167 *Msp*I	1	4	450 *Msp*I	2	4	497 *Msp*I	10	4
487 *Msp*I	3	3	474 *Msp*I	2	3	474 *Msp*I	4	3	564 *Cfo*I	1	3
85 *Msp*I	6	3							85 *Msp*I	1	3
437 *Msp*I	18	3							542 *Msp*I	2	3

Table 2. Mean relative abundance (Abund., average values as percentage) and percent contribution (Contrib., to the within-group similarity) of 18S rDNA 5′-terminal restriction fragments (T-RFs) contributing to >70 % of within-group similarity. Average similarities: Group I = 50 %; Group II = 54 %; Group III = 62 %; Group IV = 56 %

Group I			Group II			Group III			Group IV		
T-RF (bp)	Abund.	Contrib.	T-RF (bp)	Abund.	Contrib.	T-RF (bp)	Abund.	Contrib.	T-RF (bp)	Abund.	Contrib.
382 *Msp*I	29	13	382 *Msp*I	39	12	382 *Msp*I	74	12	172 *Cfo*I	16	12
196 *Cfo*I	13	11	433 *Cfo*I	41	25	172 *Cfo*I	25	9	382 *Msp*I	18	11
172 *Cfo*I	8	8	172 *Cfo*I	30	36	280 *Msp*I	24	9	433 *Cfo*I	14	11
280 *Msp*I	8	8	280 *Msp*I	13	47	433 *Cfo*I	17	8	280 *Msp*I	12	11
433 *Cfo*I	7	8	223 *Msp*I	5	54	368 *Msp*I	6	6	596 *Cfo*I	8	7
598 *Msp*I	2	5	426 *Cfo*I	2	61	419 *Cfo*I	3	5	419 *Cfo*I	3	6
600 *Cfo*I	2	5	378 *Msp*I	8	66	232 *Msp*I	5	4	279 *Cfo*I	2	6
267 *Cfo*I	1	4	199 *Cfo*I	6	71	598 *Msp*I	4	4	196 *Cfo*I	7	5
286 *Msp*I	3	4				373 *Msp*I	2	4	223 *Msp*I	1	4
430 *Cfo*I	1	4									
279 *Cfo*I	1	3									

dominant at the lower latitudes (e.g. *Msp*I-derived 439 bp peak) and those T-RFs that were dominant in sub-Arctic/Arctic regions, such as the *Cfo*I-derived 55, 90, and 93 bp T-RFs and the *Msp*I-derived 85, 88 and 437 bp T-RFs (Table 1), where *Cfo*I peaks 55, 90 and 93 showed an inverse relationship in abundance with the universally distributed 910 peak (data not shown). Several T-RFs were found solely in samples from specific biomes of the Pacific. *Cfo*I-derived T-RFs 511 and 668, and *Msp*I-derived 312 and 507 T-RFs, were either present at low frequencies or absent in high latitudinal zones, more abundant in the mid-latitudinal and equatorial waters and absent from sub-Antarctic/Antarctic waters (Fig. 6A). *Cfo*I-derived T-RFs 365, 518, 575 and 585 and *Msp*I-derived T-RFs 92 and 540 were present only in samples from the sub-Arctic/Arctic and/or sub-Antarctic/Antarctic (Fig. 6A). Distinction between the 'tropical' assemblage and all other latitudinal groupings (I, III and IV) was ascribed mainly to the presence of the *Cfo*I-derived 668 bp peak. Using the Restriction Mapper program (Version 3; www.restrictionmapper.org) representatives of the *Prochlorococcus* clade produced terminal fragment lengths of 641 to 648 when digested with *Cfo*I. This may offer a putative identification of this T-RF as a representative of the *Prochlorophytes*, as this peak is seen only in the temperate/tropical regions and coincides with the appearance of *Prochlorococcus* (~4.38×10^7 cells l^{-1}) as seen in our flow cytometry results (Fig. 3). Several less abundant T-RFs (~57 % of all T-RFs) were present sporadically and showed no observable trends with respect to latitude.

Similar patterns in eukaryotic T-RFLP profiles were apparent, although less latitudinally congruent than

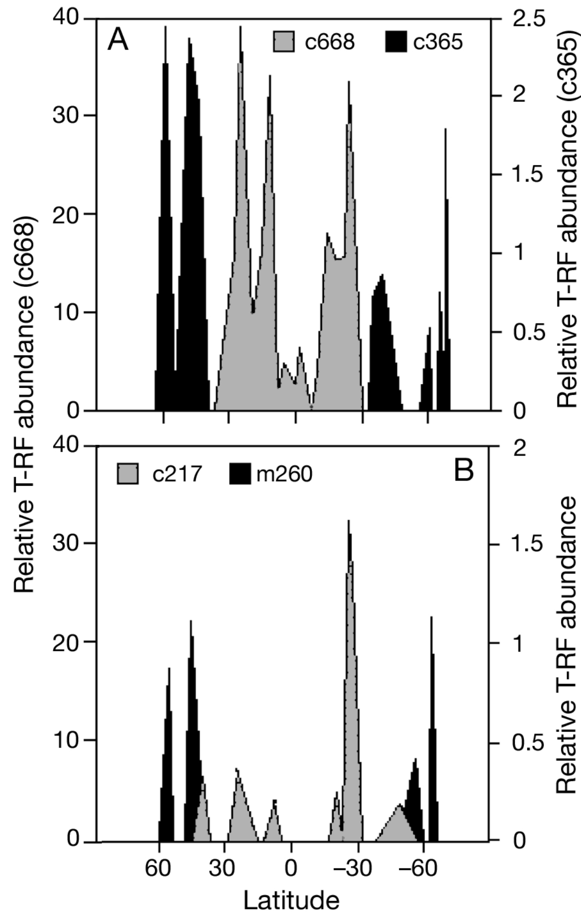

Fig. 6. Comparison of T-RFs representative of polar vs. tropical/temperate species of (A) prokaryotes and (B) eukaryotes. Other prokaryotic T-RFs *Cfo*I 511, *Msp*I 312 and 507 appeared only in temperate/tropical waters. Prokaryotic T-RFs *Cfo*I 518, 575, 585, and *Msp*I 92 and 540 appeared only in the polar latitudes. Eukaryotic T-RF *Cfo*I 226 appeared only in the polar latitudes. Eukaryotic T-RFs *Cfo*I 212, 285, 605 and *Msp*I 114, 118, and 274 appeared in temperate/tropical waters (data not shown). Positive latitude values represent Northern Hemisphere locations and negative latitudes represent the Southern Hemisphere

prokaryotic profiles. The *Cfo*I-derived 226 peak and the *Msp*I-derived 260 peak appeared only in the polar latitudes (Fig. 6B), while *Cfo*I-derived 212, 217, 285 and 605 T-RFs and *Msp*I-derived 114, 118 and 274 T-RFs appeared only in temperate/tropical regions (Fig. 6B). Several T-RFs (62% of the total T-RFs generated) contributed only a small fraction to the total peak area of a community and appeared infrequently (<3 locations).

Overall, analysis of both the prokaryote and eukaryote profiles revealed several T-RFs that were present in all 4 biogeographic regions, although sometimes represented at only 1 latitude, lending the possibility that the phylogenetic groups representing these T-RFs are indeed globally distributed. Prokaryotic profiles showed that 25% of the T-RFs generated from both *Cfo*I and *Msp*I restrictions fall within this global distribution. Eukaryotic profiles showed that 16% of *Cfo*I- and *Msp*I-generated T-RFs were globally distributed. In contrast, 8% of the prokaryotic T-RFs and 9% of the eukaryotic T-RFs constituted those groups found in only particular oceanic regions, suggesting a more endemic distribution.

DISCUSSION

Due to their small size, microorganisms are extremely abundant and disperse easily, leading some to believe that they are cosmopolitan in their distribution (Finlay 2002). Baas-Becking (1934) stated that 'everything is everywhere, and the environment selects', suggesting that local environmental conditions drive community composition. If we assume theories of cosmopolitanism to be true, then how do we explain the absence and/or presence of diverse species in the molecular analysis of microbial communities? Finlay (2002) suggests that some organisms may be extremely rare or present only in some cryptic form (e.g. cysts or spores) and hence not detected by broad detection methods (e.g. T-RFLP). In a comparison of microbial assemblages from the Arctic and Antarctic, Wilkinson (2001) proposed that universal distribution may be cell-size related, with ubiquitously distributed organisms maintaining an average size of <100 µm, suggesting that overall global species richness is moderate.

A few studies have supported the hypothesis of universal distribution of microorganisms (Finlay & Clarke 1999, Zwart et al. 2002), while others support the existence of endemic microorganisms (Whitaker et al. 2003). If microorganisms are truly endemic, our present day view of global microbial diversity and richness may be a gross underestimation. One way to determine the true distribution of microbial phylotypes is to use molecular-based methods to examine communities on large geographical scales. While by no means excluded from inherent biases (Suzuki & Giovannoni 1996), PCR-based molecular techniques offer a fast and reliable approach to assessing diversity over these large spatial scales. Utilizing T-RFLP technology, we were able to assess the diversity of the dominant portions of microbial communities encompassing a range of the Pacific Ocean from the Arctic to the Antarctic circles. Microbial diversity studies have traditionally been limited to smaller spatial scales and isolated environments, making this study unique in its scope.

T-RFLP analysis of Pacific Ocean microbial communities indicated 4 major groupings (clusters) represen-

tative of the sub-Arctic/Arctic Pacific, temperate Pacific, tropical Pacific and the sub-Antarctic/Antarctic Pacific Ocean. High within-group similarities for both prokaryotic and eukaryotic communities were driven by a relatively low number of T-RFs (8 to 14). This could be the result of several different phylogenetic clades sharing identical terminal restriction sites, resulting in the same length terminal fragment. Microbial community analysis revealed many phylogenetic groups that were ubiquitously distributed as well as those isolated to specific habitats in both prokaryotic and eukaryotic fractions. For our purposes, a ubiquitous ribotype (T-RF) is one appearing in all 4 regions of the Pacific Ocean, while an endemic ribotype is seen only in specific habitat types (e.g. the polar regions or the temperate/tropical regions). We did not observe prokaryotic populations restricted solely to the sub-Arctic/Arctic or sub-Antarctic/Antarctic, but rather populations existing in both polar regions. It has been suggested by Hollibaugh (1994) that there may be phylogenetic differences between bacterioplankton communities in response to varying types of organic matter present in the local environment due to the requirement for specialized metabolic capabilities in utilizing local substrates. It is possible that organisms may be ubiquitously dispersed throughout the Pacific, but in a non-functioning state awaiting local conditions to become favorable. In comparison to our findings of Pacific Arctic bacterioplankton communities, Ferrari & Hollibaugh (1999) analyzed samples from the Canadian Arctic using DGGE and identified 5 major clusters with similarities greater than 80%. Their findings suggested that bacterioplankton communities in this area of the Arctic are the result of responses to local environmental conditions.

Several populations of microbes were found to be restricted to the temperate and tropical latitudes, possibly in response to higher temperatures and lower nutrient resources, although we did observe a peak in nitrate at the Equatorial Upwelling zone, where we also saw an increase in eukaryotic phytoplankton (pico- and nano-), *Prochlorococcus*, and *Synechococcus* from the immediate northern waters. Distinctions between the temperate and tropical assemblages and the polar assemblages resulted from the presence of a few peaks. Prokaryotic communities were dominated by ~4 T-RFs in these regions, with the most dominant T-RF being the *Cfo*I-derived 668 bp peak. The appearance of this T-RF coincided with flow cytometry determined *Prochlorococcus* abundances observed at the temperate/tropical latitudes. Campbell et al. (1994) observed the ubiquity of *Prochlorococcus* in the latitudinal band from 40° N to 40° S in the central Pacific Ocean, where they showed that it comprises a significant portion of the photosynthetic biomass. *Synechococcus* was represented in all regions of the Pacific Ocean as shown by flow cytometry analysis. These cyanobacterial groups are an important food source for planktonic heterotrophic flagellates and ciliates (Yahel et al. 1998) and greatly contribute to the total primary production in tropical regions (Li et al. 1983). We are unable to say absolutely that the *Cfo*I-derived 668 bp T-RF is representative of *Prochlorococcus* given that T-RFLP does not allow complete identification of T-RFs, but based on preliminary computational restriction digestion with the Restriction Mapper program, we are able to relate this T-RF with *Prochlorococcus*. We were, however, unable to determine the T-RF that may be representative of *Synechococcus*. Eukaryotic mid- and lower latitude communities were dominated by ~7 T-RFs endemic to this region, of which the *Cfo*I-derived 285 bp T-RF was the most dominant.

Prokaryotic communities showed high similarity across the transect, although not all communities shared the same members throughout. This similarity may be explained by the findings of earlier investigators in that most marine bacteria belong to only 9 phylogenetic groups (Giovannoni & Rappé 2000). Eukaryotic communities also showed high similarity across the transect, but a decreased number of T-RFs were generated in comparison to prokaryotic populations. This may be due to the selection of primers used to amplify eukaryotic DNA from the mixed assemblage, under-sampling, the addition of a nuclease digestion step, or the fact that prokaryotic organisms are smaller and more abundant than eukaryotic organisms. We could hypothesize that the eukaryotic fraction contains fewer members than the prokaryotic fraction based on the ecological principle that the number of individuals in a community is inversely proportional to their size (Fenchel 1993). It is also possible that some of the prokaryotic T-RFs actually represent eukaryotic plastids. Rappé et al. (1997, 1998) reported significant numbers of eukaryotic plastids from 16S rRNA gene clone libraries off the Atlantic and Pacific coasts of the United States. It is therefore possible that the absence of pre-filtration in our samples resulted in some 16S T-RFs representing eukaryotic plastids.

The selection of restriction enzyme(s) used for T-RFLP also has a substantial effect on the resolution of the microbial community and the phylogenetic groups detected. This is due to the possibility that different phylogenetic groups may have enzyme-specific terminal-end cut sites at the same location on the PCR amplicon, resulting in the same size terminal fragment. Our selection and analysis using 2 restriction enzymes offer higher resolution than a single enzyme; however, we are aware that some T-RFs may represent more than one phylogenetic group, resulting in an underestimation of the richness of Pacific Ocean

microbial communities. Since the major goal of this study was to provide a broad spatial overview of microbial diversity in the Pacific Ocean, the use of different and/or more restriction enzymes would not necessarily increase the power of the analysis in contrast to the additional time and monetary expenditures required.

T-RFLP only recognizes the dominant members of a community, resulting in a somewhat skewed version of 'true' diversity. Low abundance members in a community may be overlooked by molecular-based methods due to sampling biases (Finlay 1998). Sampling size may influence the true diversity of a community by under-representing or excluding particular community members from small sampling sizes. Prokaryotic communities are probably less affected by our sample size (1 l) but eukaryotic organisms may require a larger sampling volume to include all representatives of the local community. Although another potential bias is the method used for DNA extraction, the protocol used was developed, in part, to improve DNA extraction from organisms which are difficult to lyse. It is assumed that our method is sufficiently robust to include all members of the microbial community (see Simmon et al. 2004), although we cannot eliminate the possibility that this method excludes the cryptic forms (e.g. spores) present as a 'seed bank', thus overlooking these phylogenetic groups which may be ubiquitously present but dormant (Finlay 2002). Although we are aware of the potential biases in our analysis, their elimination is not yet possible and will continue to be a drawback to these types of molecular approaches and large geographical surveys.

The seasonal variability along this transect may also contribute to the observed segregation of both the prokaryotes and eukaryotes into the 4 distinct biogeographical regions, as sampling in the Northern Hemisphere was completed during the late summer and Southern Hemisphere sampling occurred during the Austral spring. The results represent a 'snap shot' of the microbial diversity along a 15 400 km geographical region during a sampling time window that was as short as logistically possible.

The results herein represent a broad overview of microbial diversity on a geographic scale never before available. Discernable patterns are apparent and there is evidence that some phylotypes co-exist, while some increase in abundance only in the absence of other species. In order to obtain a more detailed view of the phylogenetic composition of these Pacific Ocean microbial communities, possibly link community structure to function, and present a more complete picture of ubiquity versus endemicism in the Pacific Ocean, cloning and sequencing must be employed, which was beyond the scope of this study.

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