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Seasonal Distributions of Dominant 16S rRNA-Defined Populations in a Hot Spring Microbial Mat Examined by Denaturing Gradient Gel Electrophoresis

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Denaturing gradient gel electrophoresis analysis of PCR-amplified 16S rRNA gene segments was used to examine the distributions of bacterial populations within a hot spring microbial mat (Octopus Spring, Yellowstone National Park). Populations at sites along the thermal gradient of the spring's effluent channel were surveyed at seasonal intervals. No shift in the thermal gradient was detected, and populations at spatially or temperature-defined sites exhibited only slight changes over the annual sampling period. A new cyanobacterial 16S rRNA sequence type was detected at temperatures from 63 to 75°C. A new green nonsulfur bacterium-like sequence type was also detected at temperatures from 53 to 62°C. Genetically unique though closely related cyanobacterial and green nonsulfur bacterium-like populations were successively distributed along the thermal gradient of the Octopus Spring effluent channel. At least two cyanobacterial populations were detected at each site; however, a limited ability to detect some cyanobacterial populations suggests that only dominant populations were observed.

We have conducted long-term studies of the composition and structure of the Octopus Spring cyanobacterial mat as a relatively simple and stable natural model system (40, 42). Realizing that an objective description of the community by using culture methods was unlikely, we developed molecular approaches and used them to demonstrate a much greater community complexity than had previously been recognized (40–42, 44–47). The molecular approaches can detect unique populations of molecules, in this case 16S rRNA (or 16S rRNA gene) sequences. One must be careful when considering whether these correspond to unique organismal populations, defined by ecologists as a group of individuals of one species (5). It is possible that an organism has multiple operons with different 16S rRNA sequences (26, 27, 29) or that molecular methods produce artifacts which do not correspond to any real organism in the community (40). The situation is further complicated by the inadequacy of the species concept for procaryotes. Species are usually defined by the ability of individuals to interbreed; however, the ecological differences distinguishing species may be the primary basis for their evolution, with sex being a mechanism ensuring isolation of gene pools (19, 24). In this sense, the term "ecotype" defined as a set of individuals within a species with a characteristic ecology (5) may be more useful. A major objective of our work was to determine the significance of populations defined by unique molecular sequences by studying their distribution relative to environmental parameters.

Distribution studies have been severely limited by the labor-intensiveness of molecular methods, which limits the number of samples that can be analyzed. Thus, the inferences that can be drawn from such a set of observations are also limited. For instance, enrichment cultures from highly diluted mat samples led to the recovery of a cyanobacterial population, *Synechococcus* sp. strain C9 (17). Although it is a numerically significant isolate, its 16S rRNA sequence has never been retrieved in cloning experiments. It was impossible to ascertain whether this inconsistency was due to the failure of molecular methods to detect the C9 population or to the very limited number of mat samples (four) used to produce clone libraries. Such a limited sample set might mask spatiotemporal variations in the dominance of *Synechococcus* populations, such as C9, in the mat. Thus, we applied a method, denaturing gradient gel electrophoresis (DGGE) analysis (16), which would allow the simultaneous detection of multiple 16S rRNA sequence types in samples, thus increasing the number of samples that could be analyzed and enabling more ecologically satisfying experimental designs (41).

Here, we used DGGE analysis to evaluate seasonal distributions of Octopus Spring microbial mat populations defined by 16S rRNA sequences along a thermal gradient. We hypothesized that the cells harboring some of the more rarely detected 16S rRNA sequence types might be seasonally dominant.

MATERIALS AND METHODS

Study site. Our study site was a hot spring microbial mat located within the southernmost effluent channel of Octopus Spring, Yellowstone National Park, Wyo. At its upstream end, the cyanobacterial mat is no longer visible at temperatures above 75°C. The water temperatures at points along the channel fluctuated by as much as 12°C over a 2-min interval. Samples were collected from sites along the effluent channel which differed in temperature range. The temperature range at each sampling site was recorded with a thermistor connected to a chart recorder. Two collection sites were marked with metal stakes so that population variation could also be examined at spatially defined as well as temperature-defined sites. This also permitted us to monitor temperature shifts in the thermal gradient that were anticipated over the course of the year.

Sample collection. The samples collected on 13 September and 19 December 1994, and 13 March and 9 June 1995 were designated the fall, winter, spring, and summer samples, respectively. All samples were collected within 2 h of solar noon. A no. 4 (7.0-mm-diameter) cork borer was inserted into the mat, and uniform cylindrical cores were removed. The top of each core, containing the entire cyanobacterial layer (approximately 1.5 mm), was sliced off with a razor blade and placed into a 1.5-ml screw-cap microcentrifuge tube. The samples were stored on dry ice during field collections. Triplicate cores were collected within areas of approximately 10 by 10 cm at intervals along the thermal gradient from 48 to 75°C. Previous DGGE studies indicated that populations within such areas are distributed homogeneously (16).

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Nucleic acid extraction, PCR, and DGGE. The methods used for nucleic acid extraction, PCR amplification, and DGGE were essentially as described previously (16). Briefly, the cells were lysed with a bead beater (Savant Instruments Inc., Farmingdale, N.Y.) at a setting of 6.5 for 45 s. Nucleic acids were extracted from the lysate with phenol-chloroform and subjected to ethanol precipitation at −20°C overnight (23). PCR amplification conditions with primers bracketing a hypervariable region of bacterial 16S rRNA genes (*Escherichia coli* positions 1055 to 1406) were as previously described (16). The primers have been shown to recover sequences from six different kingdoms in the domain *Bacteria* (16, 34). Samples (20.0 to 25.0 μl) of a PCR amplification mixture were used to visualize the multiple products that were separated by DGGE; 5.0 μl was sufficient to visualize single products that resulted from most DGGE band reamplifications (see below). Denaturing gradient gels (0.75 mm) were stained with SYBR green (Molecular Probes, Eugene, Oreg.) as specified by the manufacturer and photographed under UV transillumination.

Sequencing DGGE bands. Individual DGGE bands were excised, reamplified by PCR, and rerun on denaturing gradient gels to verify the purity of the PCR reamplification product, as previously described (16). Before being sequenced, PCR products were purified with the QIAquick Spin PCR purification kit (Qiagen Inc., Chatsworth, Calif.) as specified by the manufacturer. Automated sequencing of the purified PCR products was performed by a dedicated sequencing facility (Murdock Molecular Biology Facility, University of Montana, Missoula, Mont.) with an ABI 373A DNA sequencer (Applied Biosystems, Foster City, Calif.). Sequencing reactions in two directions were performed with a PRISM DNA dye terminator cycle-sequencing kit as specified by the manufacturer. Primers 1114F (5′GCAACGAGCGCAACCC3′) and 1368R (5′CCCGGGAAC GTATTCACCGC3′) were used in the sequencing reactions. The sequences were analyzed with Genetic Data Environment software. The identity of each band was determined by aligning its sequence with 16S rRNA sequence types previously retrieved from the Octopus Spring microbial mat. Unique sequences were defined by differences in their mobility in denaturing gradient gels and the presence of unambiguous sequence differences.

Effect of PCR template dilution or supplementation on DGGE band intensities. To aid in interpretations of DGGE profiles, the relationship between the concentrations of mat nucleic acid extract (templates) in the PCR and DGGE band intensities was examined. PCR amplifications and DGGE analyses were performed on undiluted and 0.1-, 0.09-, 0.08-, 0.07-, 0.06-, 0.05-, 0.04-, 0.03-, 0.02-, and 0.01-fold dilutions of a representative nucleic acid extract of a 59 to 67°C Octopus Spring mat sample. The undiluted sample is equivalent to those used to generate the DGGE profiles in the seasonal study.

An additional experiment that addressed the adequacy of PCR and DGGE as a means of detecting the 16S rRNA sequence of an Octopus Spring *Synechococcus* isolate, whose sequence has never been detected in situ by molecular methods, was conducted. *Synechococcus* sp. strain C9 was originally isolated from a highly diluted Octopus Spring mat enrichment (17). Cell suspensions containing 8×10^6 *Synechococcus*-shaped cells per ml were prepared from a homogenized 53 to 62°C Octopus Spring mat sample and from an 8-day broth culture of C9 cells harvested just after log-phase growth had ended. C9 cells were grown in 50 ml of medium D (10) in 250-ml culture flasks with a rotary shaking water bath incubator set at 100 rpm and 55°C. A constant irradiance of 1,200 lux was provided by cool white fluorescent lights. The cell suspensions were combined so that the C9 cells comprised 100, 90, 75, 50, and 0% of the *Synechococcus*-shaped cells in the mixtures. DNA from 1.0 ml of these suspensions was extracted, amplified by PCR, and analyzed by DGGG as described above.

Black-and-white Polaroid photographs of DGGE gels were scanned with a Scan Jet IIcx (Hewlett-Packard). The intensities of bands on the digitized images were determined, after subtraction of background, with a PowerMac 7200 (Apple, Cupertino, Calif.) and a gel analysis subroutine within the program NIH Image version 1.59 (NIH Image, written by Wayne Rasband, National Institutes of Health, Bethesda, Md., is freely available on the Internet from the anonymous FTP site zippy.nimh.nih.gov). In the case of heteroduplex DGGE bands (i.e., those consisting of one strand from each of two separate sequence types [see below]), half the band intensity was added to that of each corresponding pure-component DGGE band.

Nucleotide sequence accession numbers. The new sequences reported here have been submitted to GenBank and assigned accession number U88069 (type A″) and U90433 (type C″).

RESULTS

DGGE profiles. DGGE profiles of the Octopus Spring mat at sites along the effluent channel were obtained for each season (Fig. 1). In some cases, one of the triplicate samples did not yield PCR products and only two profiles are shown. The profiles were essentially the same for samples collected within the same site and were similar for sites at which the temperature ranges were comparable, regardless of the season. However, as the temperature range difference between sites increased, the profiles became notably different.

TABLE 1. Percent sequence similarity and number of unambiguous nucleotide differences among Octopus Spring cyanobacterial and green nonsulfur bacterium-like 16S rRNA sequence types[a]

Bacterial type	% Sequence similarity and no. of nucleotide differences for[a]:							
	A	A′	A″	B	B′	C	C′	C″
Cyanobacteria								
A		4	6	11	10			
A′	98.3		2	12	13			
A″	97.6	99.0		15	16			
B	95.9	95.5	94.8		1			
B′	96.2	95.2	94.4	99.7				
Green nonsulfur bacteria								
C							3	2
C′						99.0		2
C″						99.0	98.6	

[a] Percent similarities are below the diagonal, and unambiguous nucleotide differences are above the diagonal. The nucleotides compared correspond to *E. coli* positions 1075 to 1375, which include several highly variable regions. These percentages may differ from the true percent similarities obtained by comparing complete 16S rRNA sequences because of differences in the relative density of variable regions.

Nucleotide sequences of homoduplex DGGE bands. The identities of bands for which 16S rRNA sequences were obtained are indicated in Fig. 1 by letters which correspond to known Octopus Spring 16S rRNA sequence types. Except for sequences labelled A″ and C″, all the sequences in this study matched those previously detected in the Octopus Spring mat (16, 42). Phylogenetic analyses showed that the sequences of bands B, B′, A, A′, and A″ are cyanobacterial; those of bands C, C′, and C″ are green nonsulfur bacterium-like (46); and that of band E″ is green sulfur bacterium-like. Bands A″ and C″ were observed in previous DGGE studies but were not sequenced. They represent new cyanobacterial and green nonsulfur bacterium-like sequence types closely related to the Octopus Spring A and C types, respectively (Table 1). The 16S rRNAs of the five cyanobacterial sequence types have 16 or fewer unambiguous nucleotide differences and the 16S rRNAs of the three green nonsulfur bacterium-like sequence types have 2 to 3 unambiguous nucleotide differences among 300 positions analyzed.

Heteroduplex DGGE bands. Not all bands in the DGGE profiles corresponded to 16S rRNA molecules actually present in the mat. Several appeared to be heteroduplex molecules. These form during mixed-template PCRs when annealing occurs between similar but nonidentical products (15). A two-letter code was used to identify heteroduplex bands and their component 16S rRNA sequence types (Fig. 1). Excision and PCR amplification of heteroduplex bands yielded products that migrated to positions corresponding to their component sequences; the original heteroduplex products also reformed and migrated higher in the gradient, since base pair mismatches weaken hydrogen bonding between the double strands. For instance, PCR reamplification of the bands labelled AB′ and B′A yielded products that migrated to positions corresponding to the A and B′ bands, and reamplification of the band labelled AA′ yielded products that migrated to positions corresponding to the A and A′ bands (Fig. 2). When only one heteroduplex band is apparent (e.g., AA′), it may be that the two heteroduplex molecules migrated to the same point in the denaturing gradient gel (15). Other heteroduplex bands, BB′ and A′A″, migrated to nearly the same point and

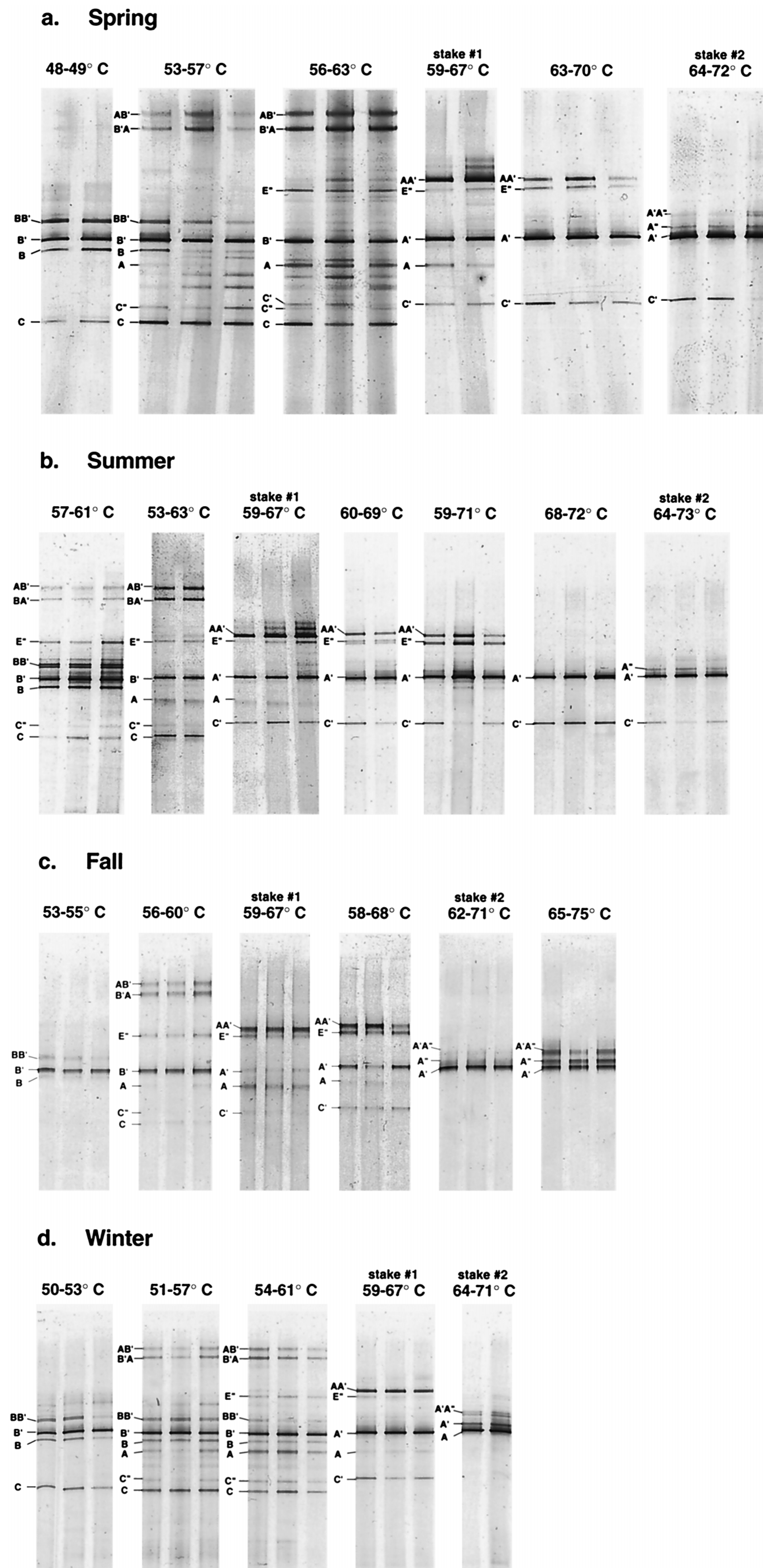

FIG. 1. DGGE profiles of PCR-amplified 16S rRNA gene segments from DNA extracted from Octopus Spring microbial mat samples at temperature-defined sites along the southernmost effluent channel during spring (a), summer (b), fall (c), and winter (d). Single letters correspond to sequence types; double letters correspond to heteroduplex bands (see the text). Sites marked by stakes are indicated.

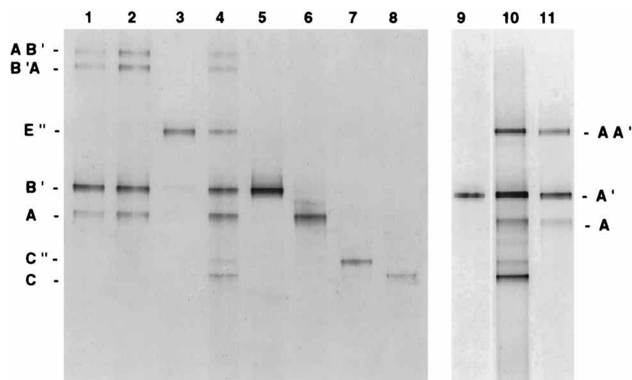

FIG. 2. Sample DGGE gel illustrating the patterns generated from excision and PCR reamplification of heteroduplex and homoduplex bands. Lanes: 4 and 10, representative Octopus Spring mat profiles from sites averaging 56 and 61°C, respectively; 1, 2, and 11, reamplified heteroduplex bands AB′, B′A, and AA′, respectively; 3 and 5 to 9, representative reamplified homoduplex bands yielding single, targeted bands E″, B′, A, C″, C, and A′, respectively.

were not always resolved. The two bands seen (Fig. 1a, 56 to 63°C sample) immediately above the C″ band in some lanes are likely to be heteroduplex molecules composed of C and C″, but this could not be completely resolved due to poor PCR reamplifications.

Distribution patterns. The temperature ranges at both marked sites, stake 1 and stake 2 (Fig. 1), were quite constant throughout the study, even though the ambient temperature was 0 and 25°C at the winter and summer sampling times, respectively. Similar sequence types were detected at these spatially defined sites. Types A, A′, C′, and E″ were detected at stake site 1 at each season. Types A′, A″, and C′ were detected at stake site 2 in spring and summer. Only type C′ was not detected in all seasons (it was absent in fall and winter [Fig. 1]). The information obtained from DGGE profiles from all sites was condensed into a diagrammatic representation of proposed distributions of Octopus Spring sequence types along the thermal gradient (Fig. 3). Because not all the sampling locations were spatially defined, the distribution patterns do not appear identical; however, overall, the order of sequence types along the thermal gradient was consistent throughout each seasonal series. The cyanobacterial types were distributed over a temperature range from 48 to 75°C in the order B, B′, A, A′, and A″. The green nonsulfur bacterium-like C and C″ types were distributed over a temperature range from approximately 48 to 64°C, and the C′ type was observed at temperatures ranging from 58 to 75°C. The green sulfur bacterium-like E″ type was distributed over a temperature range from 54 to 71°C.

Relationships between DGGE band intensities and template concentrations in the PCR. Tenfold reductions in template concentrations resulted in notable decreases in the intensities of all DGGE bands in the profile (Fig. 4a and b). Because of the sensitivity limit associated with detecting DGGE bands by staining, only the most prominent band (A′) could be quantified at 0.01- to 0.1-fold dilutions. The relationship between the template concentration and the intensity of band A′ reflects the noted decreases in intensity between each 10-fold-diluted sample (Fig. 4c) and suggests that between dilutions 0.09 and 0.01, the band intensity-to-template correspondence was roughly linear (r^2 = 0.84) (Fig. 4d).

Detection of *Synechococcus* sp. strain C9 added to mat samples. The 16S rRNA sequences of some populations detected by previous molecular or culture analyses have not been detected on denaturing gradient gels. We evaluated the ability of DGGE to detect one such population, *Synechococcus* sp. strain C9, added to a 53 to 62°C mat sample that contained cyanobacterial sequence types A and B′. The C9 16S rRNA gene segment was readily amplified from nucleic acid extracts of C9 cells, and a single band that migrated to a unique position in the denaturing gradient gel was observed (Fig. 5). The intensity of the C9 band decreased with decreasing amounts of C9 cells added to the mat sample (Fig. 5). The C9 band was barely visible when the C9 cells accounted for 50% of the *Synechococcus*-shaped cells in the sample. In contrast, the A and B′ bands, while reduced in intensity, were clearly visible even when they accounted for only 10% of the *Synechococcus*-shaped cells. Microscopy analysis indicated that the lysis efficiency of the *Synechococcus* cells was over 99%. Thus, if C9 cells and the cyanobacterial cells possessing the A and B′ sequences have equal copy numbers of the 16S rRNA gene per cell, the data suggest that there is some bias against amplification of the C9 sequence.

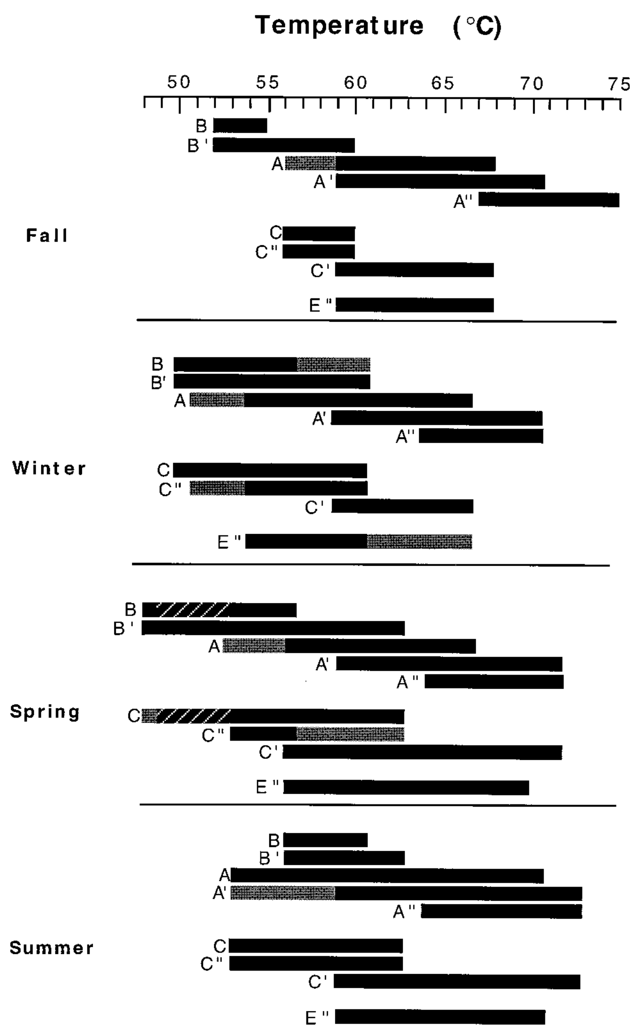

FIG. 3. Diagrammatic illustration of proposed 16S rRNA-defined population distributions based on the DGGE profiles in Fig. 1. Bars span the temperature ranges of the sites where each population was detected. Black segments indicate sites where the corresponding DGGE band was most intensely stained, gray segments indicates sites where bands appeared less intense, and striations indicate intervals over which the temperature ranges of adjacent sites did not overlap.

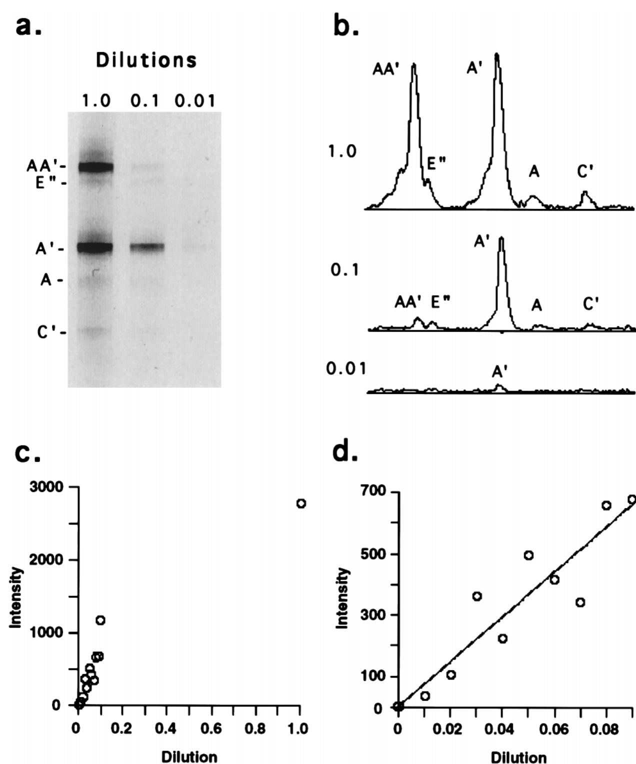

FIG. 4. Relationship between Octopus Spring mat 59 to 67°C site nucleic acid concentration and DGGE band intensity after PCR amplification. (a) DGGE profiles with sequence types indicated by letters. Lanes: 1, undiluted; 2, 0.1 dilution; 3, 0.01 dilution. (b) Digitized scan illustrating the intensities of the bands in panel a. (c) Graph of the intensity of band A′ with dilution over the entire dilution series. (d) Graph showing the intensity of band A′ in the 0.01 to 0.09 dilution range. Band intensities are in arbitrary units. The line is the result of linear regression analysis (r^2 = 0.84).

DISCUSSION

DGGE analysis provided a useful means of simultaneously detecting the spatio-temporal distribution of multiple 16S rRNA-defined populations inhabiting the Octopus Spring mat. However, the profiles had to be interpreted with caution since heteroduplex bands were also present and these do not represent unique sequence types present in the community. While the formation of heteroduplex molecules can complicate DGGE profiles, their presence can prove advantageous by providing another means of discerning homoduplex molecules that nearly comigrate in the denaturing gradient (1, 2, 13, 15, 20). For instance, the ability to discern sequence types A′ and B′ was improved by observing the presence of heteroduplex bands AB′, B′A, and AA′ (Fig. 1). Heteroduplexes were detected only among closely related sequences, suggesting that they can be expected in systems containing clusters of highly related populations.

From our cultivation and molecular evidence, we believe that the sequence types detected by DGGE were those of dominant members of the Octopus Spring mat community. For example, two different molecular methods, DGGE and cDNA cloning, indicated that type B-like cyanobacterial 16S rRNA sequences were most frequently recovered from low-temperature mat samples (40). Consistent with molecular results, type B and B′ *Synechococcus* populations were detected in enrichments inoculated with a highly diluted suspension of low-temperature mat samples containing ca. 8 *Synechococcus* cells (30, 43).

Some cyanobacterial sequences (e.g., C9, P, I, and J), which have been detected only in enrichment culture isolates or rarely in clone libraries, were never detected by DGGE. Because PCR can qualitatively bias the detection of certain 16S rRNA sequences in mixed-template reactions (14, 32, 39), we were concerned that limitations of molecular methods might explain our inability to detect these sequences. Thus, bias against the PCR amplification of the *Synechococcus* sp. strain C9 16S rRNA gene was examined. The DGGE band corresponding to C9 could be detected only if its cells were present in equivalent or greater numbers relative to other *Synechococcus*-shaped cells in the mat (Fig. 5). This might indicate that there is primer bias against amplification of the *Synechococcus* sp. strain C9 16S rRNA gene or that C9 cells possess fewer 16S rRNA gene copies than the other cyanobacteria detected in this sample (14). However, failure to detect C9 16S rRNA genes in the seasonal study suggested that its cell number never exceeded that of type A- and B-like cyanobacteria. A subdominant population level is consistent with results of dilution culture experiments suggesting that C9 cells represent between 0.4 and 4.0% of the total *Synechococcus*-shaped cells in the mat (17). There is also some evidence that C9 may be a low-light-adapted population (31). Perhaps these cells are adapted for growth at such a small percentage of the total available light energy that their population density never becomes high.

PCR amplifications can also skew results quantitatively, such that linear relationships between template concentrations and PCR products are not obeyed (39). The large amount of PCR product needed to detect DGGE bands forced us to use PCR conditions that were nonquantitative, as evidenced by the template dilution experiment (Fig. 4c). The formation of heteroduplex bands also indicated that PCR amplifications were nonquantitative as a result of interference of template-primer binding by template-template interactions (39). Therefore, we do not presume that DGGE band intensities reflect true gene abundances. We do, however, believe that the detected sequences were from dominant members of the mat community

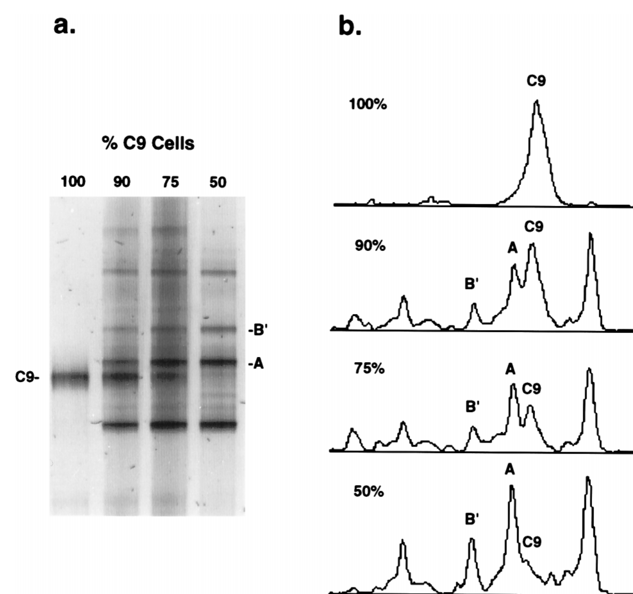

FIG. 5. DGGE profiles generated by adding different numbers of *Synechococcus* sp. strain C9 cells to a 53 to 62°C Octopus Spring mat sample prior to cell lysis, DNA extraction, and PCR amplification. (a) Gel image with labelled cyanobacterial bands A, B′, and C9. (b) Digitized scan of the image showing the relative intensities of each cyanobacterial band.

and that the appearance or disappearance of bands in DGGE profiles indicated large-scale (order-of-magnitude or greater) increases or decreases in the densities of these community members along the thermal gradient.

Temperature-dependent cyanobacterial population distributions such as we detected were predicted from the adaptations of cultivated hot spring *Synechococcus* strains (9, 28). Our results (Fig. 3) provide direct in situ evidence supporting the hypothesis that *Synechococcus* populations are optimally adapted to particular temperature ranges (7, 8). Although hybridization probe studies suggested different temperature distributions of cyanobacterial sequences A and B (33), DGGE resolved five sequence types, B, B′, A, A′, and A″, that exist at temperatures ranging from 48 to 75°C, respectively. Since temperatures at points along the effluent channel oscillate over a range of about 10°C, the upper temperature extreme at which a sequence type was detected might be greater than the highest temperature at which the population contributing it is capable of growth (10, 11).

Temperature-adapted strains of green nonsulfur bacterium-like populations were also suspected based on the pattern of temperature optimization of bacterial photosynthesis in samples collected along a thermal gradient (4). Previous hybridization probe studies detected Octopus Spring type C-like sequences over a temperature range comparable to that observed in this study, but individual C-like sequence types were not discerned (33). With the discovery of a third C-like sequence type, C″, an in situ pattern of temperature distribution emerged from DGGE analyses of this closely related group as well. C and C″ were found between about 48 and 64°C, and C′ was found between 58 and 75°C. C′ may be less prevalent at the highest-temperature regions in the fall and winter.

Clusters of closely related 16S rRNA sequences have been frequently observed in natural communities (3, 6, 12, 17, 18, 21, 24, 25, 38). These can be interpreted as evidence of numerous highly related organismal populations, multiple heterogeneous 16S rRNA operons in a more limited set of populations (26, 27, 29), or even artifacts of polymerase inaccuracy (16). Our results suggest that such clusters can indicate true variation of genetically distinct organismal populations adapted to different environmental parameters, since each molecular population exhibited a unique distribution along the thermal gradient. Populations with a characteristic ecology are defined by ecologists as ecotypes (5). The closely related *Synechococcus* and green nonsulfur bacterium-like ecotypes, defined at least in part by temperature, apparently evolved as a result of adaptive radiations. Another example of adaptive radiation has been discovered in 16S rRNA-based studies of ocean picoplankton, where closely related proteobacterium-like 16S rRNA-defined populations appear to occupy different niches in the vertical aspect of the water column (18). That different ecotypes should exhibit such little change in 16S rRNA sequence is not unexpected since the 16S rRNA gene is such a conservative genetic marker (37). 16S rRNA surveys may even underestimate the diversity of bacterial ecotypes within a system.

Light is another obvious seasonally varying parameter that might influence community structure in temperate habitats. At the two defined sites, stakes 1 and 2 (Fig. 1), the temperature range remained constant seasonally. However, the average daily solar radiation in December is typically 25% of that in June (data from the National Renewable Energy Laboratory, horizontal flat plate collector, Sheridan, Wyo., station, latitude 44°77′N, longitude 106°97′W, elevation, 1,209 m; available on-line at http://solstice.crest.org.). The stability in community composition at stake site 1 is consistent with $^{14}CO_2$ labelling experiments which indicated that neither cyanobacterial nor bacterial photosynthesis within the Octopus Spring mat was limited by a 73% reduction in light intensity (22). In addition, oligonucleotide hybridization probe studies at a 50°C site revealed no decrease in the 16S rRNA of B-type cyanobacterial populations after a 93% reduction in light intensity for 1 week (33). However, the absence of population C′ from the fall and winter samples at the stake 2 site might indicate that light availability does play a role in the ability of this population to inhabit the highest-temperature extremes of the mat.

The measured seasonal stability in cyanobacterial populations contrasts with the observation of sun- and shade-adapted (summer and winter) ecotypes of *Plectonema notatum*, described as seasonally dominant populations in another temperate hot spring mat community (35, 36). We cannot, however, exclude the possibility that the Octopus Spring mat populations are vertically structured or light adapted, especially since multiple cyanobacterial populations were detected at each site. It is also possible that more subtle changes in the densities of observed populations were not detected or that density changes occurred among undetected, possibly subdominant populations.

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