

Distribution of Cultivated and Uncultivated Cyanobacteria and *Chloroflexus*-Like Bacteria in Hot Spring Microbial Mats

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Oligodeoxynucleotide hybridization probes were developed to complement specific regions of the small subunit (SSU) rRNA sequences of cultivated and uncultivated cyanobacteria and *Chloroflexus*-like bacteria, which inhabit hot spring microbial mats. The probes were used to investigate the natural distribution of SSU rRNAs from these species in mats of Yellowstone hot springs of different temperatures and pHs as well as changes in SSU rRNA distribution resulting from 1-week in situ shifts in temperature, pH, and light intensity. *Synechococcus lividus* Y-7c-s SSU rRNA was detected only in the mat of a slightly acid spring, from which it may have been initially isolated, or when samples from a more alkaline spring were incubated in the more acid spring. *Chloroflexus aurantiacus* Y-400-fl SSU rRNA was detected only in a high-temperature mat sample from the alkaline Octopus Spring or when lower-temperature samples from this mat were incubated at the high-temperature site. SSU rRNAs of uncultivated species were more widely distributed. Temperature distributions and responses to in situ temperature shifts suggested that some of the uncultivated cyanobacteria might be adapted to high-, moderate-, and low-temperature ranges whereas an uncultivated *Chloroflexus*-like bacterium appears to have broad temperature tolerance. SSU rRNAs of all uncultivated species inhabiting a 48 to 51°C Octopus Spring mat site were most abundant in the upper 1 mm and were not detected below a 2.5- to 3.5-mm depth, a finding consistent with their possible phototrophic nature. However, the effects of light intensity reduction on these SSU rRNAs were variable, indicating the difficulty of demonstrating a phototrophic phenotype in light reduction experiments.

The use of small subunit (SSU) rRNA as a marker for genetically distinct members of natural microbial communities has revealed that our knowledge of community composition as assayed by microscopic and culture methods is very poor (9, 10, 12, 22, 31). For example, in the course of molecular characterization of a model geothermal community, the 50 to 55°C Octopus Spring cyanobacterial mat, we have discovered numerous SSU rRNA sequences from uncultivated species but none from any species which has been cultivated from this type of habitat (27, 30, 31). Phylogenetic characterization of many of these SSU rRNAs suggests that several uncultivated cyanobacteria (sequence types A, B, I, and J) and at least one uncultivated phylogenetic relative of the green nonsulfur bacterium *Chloroflexus aurantiacus* (sequence type C) inhabit the mat (33, 34). But, SSU rRNA sequences of the cultivated thermophilic cyanobacterium *Synechococcus lividus* and green nonsulfur bacterium *C. aurantiacus*, both of which are thought to be important in formation of this mat community (8, 29), have so far not been recovered. The basis for the incongruence between cultivated and naturally occurring species, and for the presence of genetically diverse cyanobacterial and green nonsulfur bacterial species in a single community, is not known. We used the sequence data for these SSU rRNAs to design specific oligodeoxynucleotide hybridization probes, so that we could begin to address these questions. We studied the distributions of SSU rRNAs contributed by these species in the Octopus Spring cyanobacterial mat and in other microbial mats found in hot springs of different temperatures and pHs.

We also explored the phenotypes of these species, as revealed by changes in their SSU rRNAs in response to varying environmental parameters.

MATERIALS AND METHODS

Probes. Oligodeoxynucleotide probes specific for individual SSU rRNAs (Table 1) were designed to complement unique sequences within a hypervariable region, by following the general approach outlined by Stahl and coworkers (24, 25). A probe designed to complement a universally conserved region of eubacterial and archaeobacterial SSU rRNA sequences was used to measure total SSU rRNA. SSU rRNA of eucaryotic microorganisms was not expected on the basis of the lack of microscopic evidence of eucaryotes and the lack of lipid biomarkers which distinguish eucaryotes in most of these mats (28). Probes (synthesized by American Synthesis, Inc., Pleasanton, Calif.) were ³²P labeled by using T4 polynucleotide kinase (Promega) and purified by polyacrylamide gel electrophoresis, according to standard protocols (21). Radiolabeled probes, visualized either by ³²P excitation of an intensifying screen or after autoradiography, were excised from the gel, eluted into distilled water, filtered to remove gel particles, and stored at -20°C.

Cultures and mat samples. *S. lividus* Y-7c-s, a gift from R. W. Castenholz (University of Oregon), was grown at 54°C as described in reference 1. A frozen cell pellet of *C. aurantiacus* Y-400-fl was a gift from M. T. Madigan (Southern Illinois University). Transformants of *Escherichia coli* Q358 containing plasmid vectors with cloned inserts representing SSU rRNA sequence types of uncultivated Octopus Spring mat inhabitants were from our own collection. These were grown at 37°C in Luria-Bertani broth (20) containing either 12.5 µg of tetracycline (pBR322) or 50 µg of ampicillin (pGEM) per ml.

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TABLE 1. Characteristics of deoxynucleotide probes

Target organism	Region ^a	Probe sequence ^b	T_m ^c (°C)	T_H ^d (°C)
Type A	1254–1275	5'TCCCGCTCGCGCTTCGCTGCC	78	68
Type B	1254–1275	5'TCACGCTCGCGCTTCGCGACC	78	68
Type I	1254–1275	5'TTCGTCTCGCGAGCTTGCAACC	70	58
Type J	1240–1281	5'ATTATCCCTCTGTCCACGCCA	65	54
<i>S. lividus</i> Y-7c-s	1273–1294	5'CGTGGTTAAGAGATTAGCTCG	64	54
Type C	1273–1294	5'SCCCSTTT-GGCGATTGCGATC	67	58
<i>C. aurantiacus</i> Y-400-fl	1273–1294	5'CCACGTTTAGCGATTAGTTCC	66	58
Universal ^e	519–536	5'GTATTACCGCGGCKGCTG	59	47

^a *E. coli* position numbers (11).

^b International Union of Pure and Applied Chemists abbreviations.

^c Melting temperature (T_m) = 2°C (A+T) + 4°C (G+C) (15).

^d The hybridization temperature (T_H) was determined empirically. See the text for hybridization conditions.

^e Modified from reference 25.

Mat samples were obtained from hot springs in Yellowstone National Park, Wyoming. The temperatures and pHs of the sampling sites in these springs are given in Table 2 and Fig. 2. The location of Nymph Creek is given in reference 32. Locations of Octopus Spring, Mushroom Spring, and Twin Butte Vista Spring are given in reference 5. The Clearwater Springs group is located just west of the Grand Loop road ca. 7.5 km north of Norris Junction. Site A was an oval-shaped pool (diameter, ca. 2 m) first encountered along the path leading from a parking turnout just south of the group of springs; a luxuriant cyanobacterial mat covered the bottom of this pool. Site B was one of several tiny (diameter, ca. 0.3 m) pools with only 1 to 2 cm of water overlying a thin cyanobacterial mat, located in the grasses just to the east of site A. Site C was within the effluent channel of a large source pool without cyanobacterial growth, itself north of site A and the source of the most southerly effluent channel in the Clearwater Springs group. Site D was within the other major effluent channel of the Clearwater Springs group, which originates from springs yet more northerly and which flows north around a mound separating the two major effluents. Sites C and D were ca. 1 to 3 m upstream from the confluences of the effluent channels with Obsidian Creek. Site E was a small pool (diameter, ca. 1 m) within the grasses immediately east of the source pool of the southernmost effluent channel mentioned above. Site E contained flocculent cyanobacterial growth which accumulated near the water surface.

Most samples for distribution analysis were 415.5-mm² cores (no. 15 cork borer) containing the top 5 mm (the bioactive layer as defined in reference 29). Larger cores (diameter, 44 mm; 1,520 mm²) were collected at a 48 to 51°C site in the Octopus Spring mat and were carefully sectioned along natural laminations by using a flame-sterilized spatula at approximately 1- to 3-mm intervals for vertical profile analysis. Parallel samples were used to measure chlorophylls by in vivo absorption spectroscopy of mat homogenates suspended in 55% sucrose (26). Some hot springs contained less-thick and/or -cohesive cyanobacterial accumulations, which were sampled by scraping biomass with a spatula (Nymph Creek and Clearwater Springs site B) or by aspiration with a syringe (Clearwater Springs site E).

Temperature shift experiments were performed by transferring cores (no. 6 cork borer; 113 mm²) between Octopus Spring 50°C and 63 to 67°C sites for a 1-week in situ incubation. Cores from the sampling site were contained within 2-dram (7.4-ml) glass vials left open to permit exposure to water flowing above the mat at the incubation site. A duplicate set of cores was incubated at the collection site as controls. pH

shift experiments were performed in a like manner by transferring cores (113 mm²) between Clearwater Springs sites A and D for a 1-week in situ incubation. It was impossible to locate sites where only pH varied, so the shift included a temperature change as well as a pH change (site A, 54°C, pH 6.2; site D, 64°C, pH 7.8). Light reduction experiments were performed in situ by covering the 48 to 51°C Octopus Spring mat for 1 week with muslin or black plastic screens sufficient to reduce light intensity by 93 and 100%, respectively (14). Control samples were removed before and after the period of covering for comparison with samples which had experienced 1 week of reduced natural light intensity. During the mid-August incubation period, natural midday light intensity without cloud cover was 2,150 microeinsteins m⁻² s⁻¹. This experimental design permitted de novo colonization as well as population shifts within samples, but obvious evidence of new mat growth was observed only in the experiments conducted at Clearwater Springs. For these samples, we removed thin veils of mats which had grown across the tops of the sample vials.

After collection, all samples were immediately frozen in liquid nitrogen and were transported on dry ice to the laboratory, where they were kept at -70°C until being processed.

Extraction and purification of target nucleic acids. Plasmids from transformants were purified by using a Qiagen <Plasmid> kit according to the instructions provided by the manufacturer (Qiagen Inc., Studio City, Calif.). All other frozen pellets or samples were thawed by resuspension at 50°C in lysis buffer (80 mM NaCl, 8 mM Tris-HCl [pH 7.6], 0.8 mM EDTA, 50 mg of lysozyme [Sigma Chemical Co.]) and were homogenized in a Dounce tissue homogenizer before lysis in a French pressure cell (three passages at 20,000 lb/in²). After lysis, 1 ml of proteinase digest buffer (2.5 M NaCl, 5% sodium dodecyl sulfate [SDS]) was added, and the samples were digested with 2 mg of proteinase K (Sigma) for 1 h at 50°C. Nucleic acids were purified by phenol, phenol-chloroform (1:1), and chloroform extractions, followed by ethanol precipitation, by using standard methods (21). DNA was removed by using RQ1 DNase according to the manufacturer's protocol (Promega Corp., Madison, Wis.). DNA and RNA concentrations were estimated by absorption spectrometry. The purified RNA was between 120 and 1,540 nucleotides in length, ensuring a low probability that target sites were sheared. This lysis method was superior to several other methods (freeze-thaw combined with proteinase K digestion as described above, Bead-Beater [2 min in the presence of buffer-saturated phenol with 0.1-mm-diameter glass beads], guanidinium isothiocyanate [6], and combinations of guanidinium isothiocyanate and French press procedures) in terms of RNA yield (20).

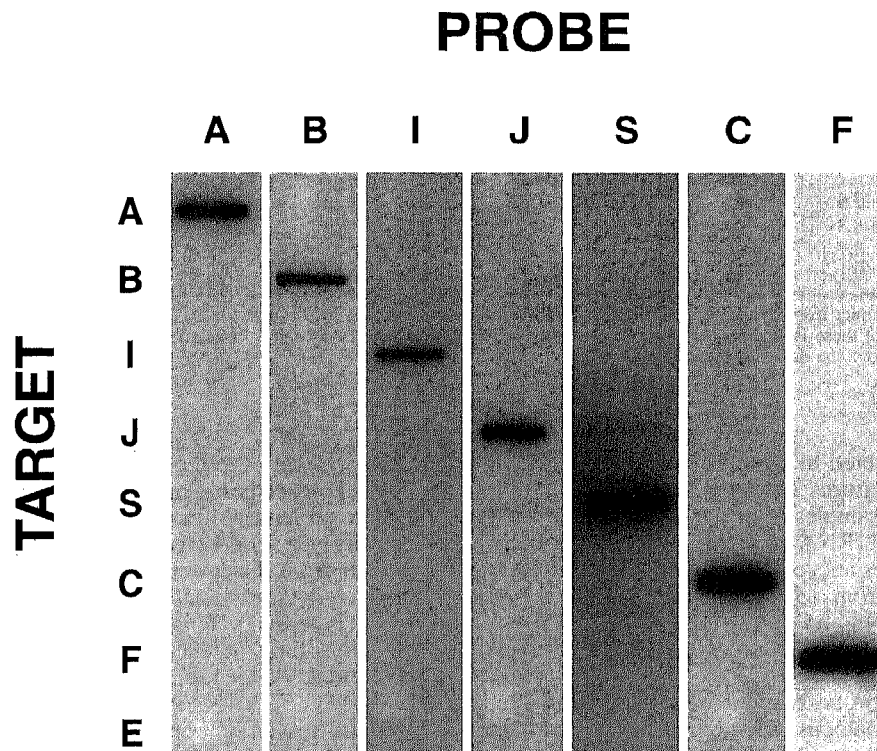


FIG. 1. Specificities of oligodeoxynucleotide probes used in this study. The probes are described in Table 1. Targets: A, B, I, and J, cyanobacterial SSU rDNA clones (cDNA clones coding for rRNA) retrieved from the Octopus Spring mat; S, *S. lividus* RNA; C, *Chloroflexus*-like SSU rDNA clone retrieved from the Octopus Spring mat; F, *C. aurantiacus* RNA; E, *E. coli* RNA.

Hybridization reactions. RNA targets were denatured by incubation for 15 min at 65°C in 5× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaH₂PO₄, 1 mM EDTA) containing 7.5% formaldehyde. DNA targets were denatured by incubation for 1 h at 65°C in 1 N NaOH. Target nucleic acids (1 pmol) were then applied to a 0.1-μm Nytran filter (Schleicher & Schuell, Keene, N.H.) contained within a Minifold II slot blot device (Schleicher & Schuell). Wells were rinsed with 5× SSPE before and after application of the target. The filter was baked for 30 min at 80°C, and target nucleic acids were cross-linked to the filter by exposure to UV light (16) in a germicidal hood for 10 min. Filters were prehybridized for 2 h at the temperature of hybridization (Table 1) in 250 μl of hybridization buffer [5× SSPE, 0.5 mg of poly(A) per ml, 0.1 mg of *E. coli* tRNA (Sigma) per ml, 10× Denhardt's solution (21), 0.1% SDS] per cm² of filter. After addition of 30 to 40 pmol of radiolabeled probe, filters were hybridized at the temperature of hybridization for 10 to 12 h. Filters were then washed three times at the temperature of hybridization in 2.5× SSPE containing 0.1% SDS, dried at room temperature, and used to expose Kodak X-Omat AR film for a period sufficient to provide images between 0.1 and 1.0 A₅₄₀ units to ensure film linearity (21).

Analysis of results. The extent of probe reaction was quantified by scanning autoradiograms with a GS-300 scanning densitometer (Hoefer Scientific Instruments, San Francisco, Calif.) and integrating peaks (GS-350 DataSystem, Hoefer Scientific Instruments). Specific probe reactivity (integration units) was normalized by the universal probe response measured on identical samples to determine a relative probe response. All samples used for comparative analysis were quantified by using the same autoradiogram. To evaluate extraction efficiency or vertical distribution, estimates of total

RNA (based on absorbance) or SSU rRNA (based on the universal probe response in integration units) per subsample were converted to amounts per whole sample. This was done by dividing the amount per subsample by the proportion of the whole sample the subsample represented. Differences in means of triplicate mat samples were tested by using a paired Student's *t* test (Sigmaplot; Jandel Scientific).

RESULTS

The specificities of probes are illustrated in Fig. 1. Probe response was linear with target RNA or DNA concentration up to 1.0 pmol. RNA was not available for uncultivated species, so we could not measure absolute probe responses. By dividing specific probe responses by the response of the universal probe, we could determine a relative probe response independently of differences in RNA concentration among samples. Without standardization, we were unable to compare the reactivities of different probes (even in the same sample), but it was possible to compare relative responses for the same probe in different samples.

Natural distribution of SSU rRNAs. Probe reactions with RNAs extracted from samples of mats in hot springs of different temperatures and pHs are shown in Table 2 and in Fig. 2, which exhibits temperature distribution in the Octopus Spring mat (pH here varied only between 8.3 and 8.7). The *S. lividus* probe reacted only with RNA from the Clearwater Springs site A mat. Probes against uncultivated cyanobacteria showed much broader reactivities with RNAs extracted from mats and biomass in springs of different temperatures and pHs, with the exception that the probe complementing the type I cyanobacterial SSU rRNA did not react with any sample. Type

TABLE 2. Relative responses of hybridization probes with rRNAs extracted from hot spring microbial mats^a

Sample	pH	Temp (°C)	Mean relative response with the following probe ± SE ^b :					
			Type A	Type B	Type J	<i>S. lividus</i>	Type C	
Clearwater Springs								
Site A	6.2	54	—	0.55 ± 0.17	—	0.64 ± 0.10	—	—
Site C	6.7	65	4.73 ± 0.29	—	—	—	—	—
Site D	7.8	64	1.70 ± 0.15	1.86 ± 0.25	—	—	—	—
Mushroom Spring	8.3	54	—	2.52 ± 0.20	—	—	—	2.43 ± 0.20
Octopus Spring	8.7	48–51	0.16 ± 0.02	0.68 ± 0.03	1.73 ± 0.08	—	—	0.37 ± 0.21
Twin Butte Vista	9.1	62	—	1.54 ± 0.17	0.55 ± 0.09	—	—	1.22 ± 0.21

^a No probe reacted with samples from Nymph Creek (pH 2.8, 47°C) or Clearwater Springs sites E (pH 5.0, 50°C) and B (pH 5.4, 45 to 51°C). The *C. aurantiacus* probe did not react with any of these RNA samples. The samples were collected between 31 July and 8 August 1991.

^b *n* = 3. —, no response above the detection limit.

A SSU rRNA was detected in samples from springs with pH 6.7 to 8.7 and temperatures between 48 and 70°C. It was especially abundant in samples at or above 60°C and was the only cyanobacterial SSU rRNA for which a probe response was observed in mat samples above 67°C. Type B SSU rRNA was detected in samples from springs of pH 6.2 to 9.1 and temperatures between 48 and 67°C. Type J SSU rRNA was detected in samples from springs of pH 8.5 to 9.1 with temperatures between 48 and 62°C.

The *C. aurantiacus* probe reacted only with RNAs from samples of a single Octopus Spring mat site, where the temperature varied between 61 and 70°C (Fig. 2). The type C probe reacted with samples from springs of pH 8.3 to 9.1 and temperature between 48 and 70°C (Table 2 and Fig. 2). The relative abundance of type C SSU rRNA along the Octopus Spring mat thermal gradient was greater above 60°C.

None of the specific probes reacted with RNAs extracted from mats of pH 2.8 to 5.4 (Table 2). Although the Nymph Creek algal mat is devoid of cyanobacteria (5, 32), Clearwater Springs sites B and E contained cyanobacteria, and those in site E were unicellular.

The vertical distributions of total SSU rRNA and of SSU rRNAs reactive with probes against sequence types A, B, J, and C (i.e., those existing naturally at the 48 to 51°C Octopus Spring mat site) are shown in Fig. 3. Total SSU rRNA was most abundant in the uppermost mat layer and was detected in decreasing amounts in deeper layers to a depth of 7 to 10 mm. Type A, B, J, and C SSU rRNAs were most abundant in the uppermost 1 mm of the mat, decreased with depth, and were not detected below a depth of 2.5 (type J) to 3.5 mm (types A to C). Chlorophyll *a* and bacteriochlorophyll *a* were in highest concentrations in the upper 1 mm and decreased with depth, but bacteriochlorophyll *c* was most concentrated in the 1- to 2.5-mm depth interval (data not shown).

Distribution of SSU rRNAs after in situ perturbation. The natural distributions of type A, B, and J SSU rRNAs suggest that the cyanobacteria contributing these sequences may be distributed toward high-, moderate-, and low-temperature regions of mats, respectively (Fig. 2). Possible adaptation of cyanobacterial sequence types A, B, and J to specific temperature ranges was further suggested by the results of temperature shift experiments with Octopus Spring mat samples (Fig. 4). A shift of samples collected at a 50°C site to a 63 to 67°C site resulted in a reduction of type B and J probe responses to below detection limits ($P < 0.01$) but an increase in type A probe response from below its detection limit ($P < 0.01$). A shift of samples collected at the higher-temperature site to the lower-temperature site had less effect on cyanobacterial SSU rRNA distribution when the responses of shifted samples are

compared with those of controls which were incubated in situ. The difference between in situ and time zero controls suggests that some natural changes in cyanobacterial SSU rRNA (especially type A) occurred during the 1-week in situ incubation. A shift from the lower to the higher temperature caused an increase in *C. aurantiacus* SSU rRNA from below its detection limit ($P < 0.01$). *C. aurantiacus* SSU rRNA was below the detection limit in 63 to 67°C samples under all incubation conditions. Temperature shifts did not change the distribution of type C SSU rRNA.

Shifts of samples between Clearwater Springs sites A and D involved changes in both temperature and pH. Although changes in the relative amounts of SSU rRNAs occurred

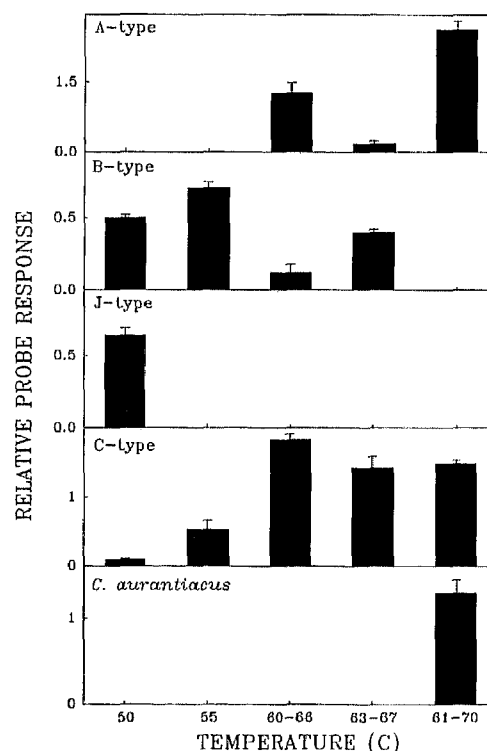


FIG. 2. Temperature distribution of cyanobacterial (types A, B, and J), *C. aurantiacus* Y-400-fl, and *Chloroflexus*-like (type C) SSU rRNAs in the Octopus Spring cyanobacterial mat, as measured by responses of specific probes relative to the universal oligonucleotide hybridization probe. Bars, standard errors ($n = 3$). Samples were collected on 19 September 1991.

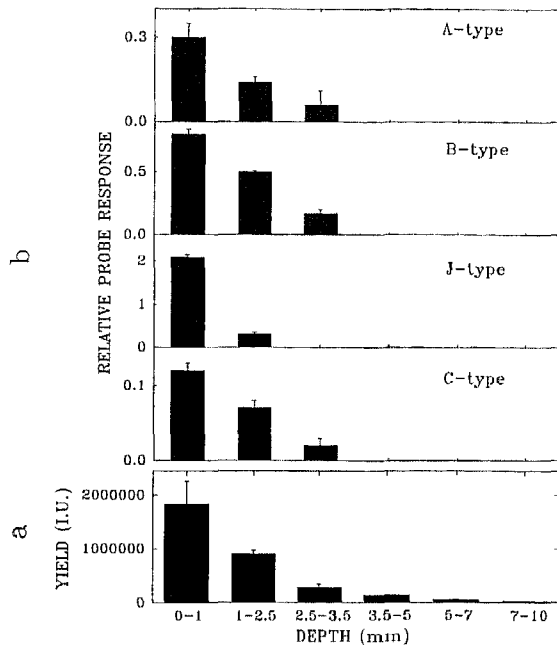


FIG. 3. Vertical distribution of SSU rRNAs in the Octopus Spring 48 to 51°C mat, as measured by oligodeoxynucleotide hybridization probe responses. (a) Response of the universal probe. I.U., integration units. (b) Responses of probes specific for cyanobacterial (types A, B, and J) and *Chloroflexus*-like (type C) SSU rRNAs relative to the universal probe. Bars, standard errors ($n = 3$). Samples were collected on 8 August 1991.

between time zero and the end of the in situ incubation, the same sequence types were present in both controls (e.g., type A was absent but type B and *S. lividus* were present at site A, and types A and B were present but *S. lividus* was absent at site D [Fig. 5]). A shift from lower temperature and pH (site A; 54°C and pH 6.2) to higher temperature and pH (site D; 64°C and pH 7.8) resulted in a decrease in the amount of *S. lividus* SSU rRNA to below detection limits ($P < 0.05$). The rise in the amount of type A SSU rRNA from below the detection limit in controls ($P < 0.01$) and the decrease in the amount of type B SSU rRNA (relative to the in situ control; $P < 0.05$) were consistent with the changes observed in the temperature upshift experiment with the Octopus Spring mat (Fig. 4). The shift from high temperature and pH to low temperature and pH resulted in a rise in the amount of *S. lividus* SSU rRNA from below detection limits ($P < 0.05$). Changes in the amounts of type A and type B SSU rRNAs between time zero and the end of the in situ incubation exceeded and thus obscured changes due to shifting between sites.

In situ light intensity reduction had variable effects on the abundance of SSU rRNAs of the sequence types found in the Octopus Spring 40 to 51°C mat (Fig. 6). Reduction of light did not significantly alter the relative probe reactions with type B and type C SSU rRNAs compared with the time zero controls ($P > 0.05$). The amount of type J SSU rRNA was significantly smaller than that of the controls ($P < 0.05$) after in situ decreases in ambient light intensity of 93 and 100%. Type A SSU rRNA was weakly detected in the time zero samples; disappearance due to light reduction was obscured by the fact that type A SSU rRNA was also not detected in the in situ controls (data not shown).

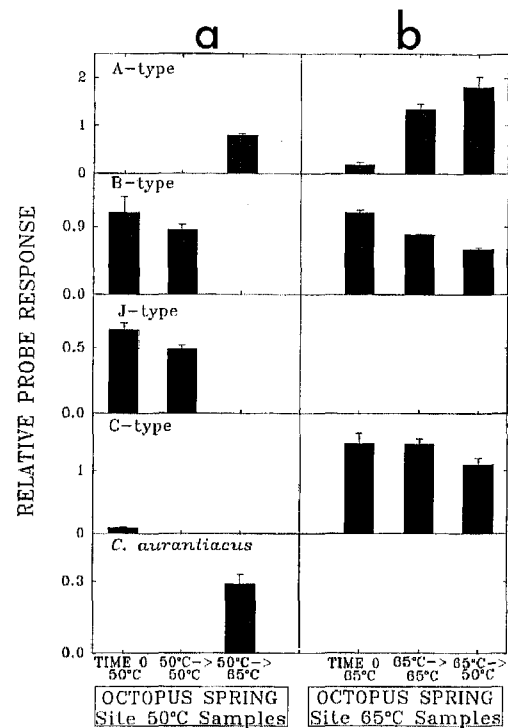


FIG. 4. Effects of shifting Octopus Spring mat samples collected at 50°C to 63 to 67°C (50→65) (a) and samples collected at 63 to 67°C to 50°C (65→50) (b) on cyanobacterial (types A, B, and J), *C. aurantiacus*, and *Chloroflexus*-like (type C) SSU rRNAs, as measured by responses of specific probes relative to the universal oligonucleotide hybridization probe. Control samples were incubated at the collection site (50→50 and 65→65). Results after a 1-week in situ incubation are compared with those for samples collected before incubation (time zero). Bars, standard errors ($n = 3$). The experiment was conducted between 19 and 26 September 1991.

DISCUSSION

Our results suggest that individual phototrophic species are adapted to specific environments within hot spring microbial mats. This specialization may help explain the failure of cloning and sequencing efforts so far to recover SSU rRNAs of *S. lividus* and *C. aurantiacus* from the 50 to 55°C Octopus Spring cyanobacterial mat (27, 30, 31), as these species appear to be less abundant in samples from this location. Species in lower abundance should contribute less to the total SSU rRNA pool and are thus less likely to be detected by methods that retrieve SSU rRNA sequences. Specialization may also increase diversity, explaining why different cyanobacterial and *Chloroflexus*-like SSU rRNAs have been observed in a single mat community. Below, we consider the possible specialization of the cultivated and uncultivated phototrophic species we studied to different environmental parameters.

S. lividus Y-7c-s SSU rRNA was directly detected only in the slightly acid Clearwater Springs site A, which is likely to have been the source pool from which this strain was initially cultivated (13). Although pH has been considered as a variable controlling the distribution of thermophilic cyanobacteria in general (4), it may also be important in controlling diversity among unicellular thermophilic cyanobacteria. In this regard, it is interesting that unicellular cyanobacteria also inhabited Clearwater Springs site E (pH 5.0) but RNA extracted from this cyanobacterial biomass did not react with any of our

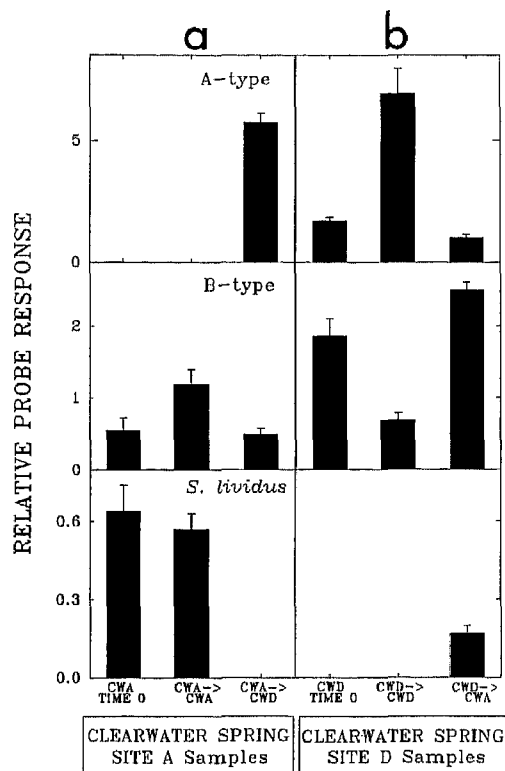


FIG. 5. Effects of shifting Clearwater Springs mat samples collected at site A (54°C, pH 6.2) to site D (64°C, pH 7.8) (CWA→CWD) (a) and samples collected at site D to site A (CWD→CWA) (b) on cyanobacterial (types A and B) and *S. lividus* SSU rRNAs, as measured by responses of specific probes relative to the universal oligonucleotide hybridization probe. Controls were incubated at the collection site (CWA→CWA and CWD→CWD). Results after a 1-week in situ incubation are compared with those for mat samples collected before incubation (time zero). Bars, standard errors ($n = 3$). The experiment was conducted between 31 July and 8 August 1991.

cyanobacterial probes. This suggests that other uncultivated thermophilic unicellular cyanobacteria exist at a yet lower pH. Although *S. lividus* SSU rRNA was not directly detected in other mats, it was detected after the Clearwater Springs site D mat (pH 7.8, 64°C) was shifted to site A, the pH 6.2, 54°C pool in which *S. lividus* SSU rRNA was detected. This could have been due to overgrowth of cyanobacteria occurring within site A or to an increase in a small, undetectable *S. lividus* population native to the site D mat. We have also been able to detect *S. lividus* SSU rRNA in enrichment cultures inoculated with Octopus Spring 50°C mat samples, so long as the inoculum was not first highly diluted (20). This provides direct evidence for the presence of *S. lividus* at a low population density in more alkaline mats and suggests that the range of environments in which *S. lividus* can survive is broader than is indicated by direct hybridization probing of SSU rRNA. The relatively greater abundance of *S. lividus* in the Clearwater Springs site A mat is apparently due to the locally greater competitiveness of this species (possibly because of a lower pH).

C. aurantiacus Y-400- β SSU rRNA was detected only in the 61 to 70°C Octopus Spring mat samples and after 50°C Octopus Spring mat samples were shifted to higher temperatures. This suggests that this isolate may be a high-temperature-adapted strain. The existence of *Chloroflexus* strains adapted to narrow temperature ranges was suggested by the

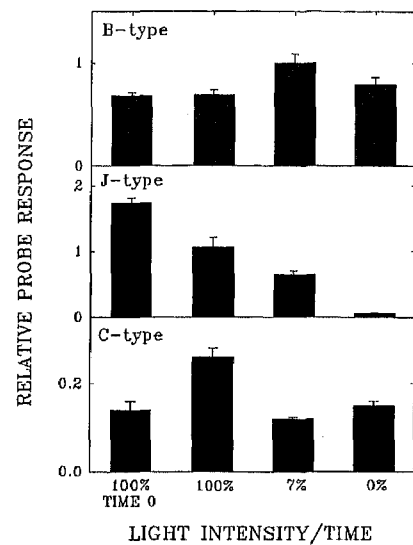


FIG. 6. Effects of reducing light intensity to 7 or 0% of full natural light (100%) on cyanobacterial (types B and J) and *Chloroflexus*-like (type C) SSU rRNAs in 48 to 51°C Octopus Spring mat samples, as measured by responses of specific probes relative to the universal oligonucleotide hybridization probe. Results after a 1-week in situ incubation are compared with those for mat samples collected before incubation (time zero). Bars, standard errors ($n = 3$). The experiment was conducted between 8 and 16 August 1991.

physiological ecology experiments of Bauld and Brock (2). In the temperature shift experiment, no visible evidence of colonization was observed, suggesting that the rise of *C. aurantiacus* SSU rRNA was due to an increase in the density of a population indigenous to the 50°C mat, which was initially below our probe detection limit. The existence of high-temperature-adapted strains as allochthonous inhabitants of lower-temperature regions of the mat is consistent with downstream dispersal and survival at a suboptimal temperature.

The phenotypic specialization of some uncultivated phototrophic species may also be revealed by some of our results. For instance, both natural distribution and temperature shift results suggest that type A, B, and J SSU rRNAs may be contributed by high-, moderate-, and low-temperature-adapted cyanobacteria, and temperature-adapted *Synechococcus* strains were shown to exist in an Oregon hot spring cyanobacterial mat (17). Further, photosynthesis in the Octopus Spring mat was shown to be adapted to the temperature at the site of sample collection (3). As argued for *C. aurantiacus* above, strains adapted to high temperatures would likely be distributed to and survive at a low population density in cooler downstream regions of the mat (e.g., type A SSU rRNA would be present below the detection limit at a low temperature and become more abundant upon a temperature upshift). However, the distribution to and survival of low-temperature-adapted strains in higher-temperature mat regions may be prevented by flow and/or a lethal temperature (e.g., type J SSU rRNA would not be present at a high temperature and thus could not become more abundant upon a temperature downshift). The effect of temperatures above the upper limit may be more severe than the effect of temperatures below the lower limit (e.g., disappearance of type B and J SSU rRNAs upon a temperature upshift but persistence of type A and B SSU rRNAs upon a temperature downshift).

The localization of type A, B, J, and C SSU rRNAs within or

near the 1- to 2-mm thick photic zone of the mat (8) is consistent with the possible phototrophic nature of the species they represent. However, differences in vertical distribution and in the results of light shift experiments suggest different types of adaptation to light. For instance, the SSU rRNA most localized toward the mat surface (type J) appears to be contributed by a cyanobacterium which is obligately light dependent. In contrast, the type B SSU rRNA was detected in layers beneath the photic zone and did not exhibit sensitivity to light. This SSU rRNA might be contributed by a cyanobacterium which is able to survive and grow in darkness or at a very low light intensity, if light leakage occurred during our experiment. Some hot spring cyanobacteria are known to carry out fermentative metabolism (19), which could permit growth in darkness. Cyanobacterial strains adapted to different light intensities are also known to exist in some hot spring microbial mats (23). The type C SSU rRNA, like the type B SSU rRNA, was detected below the photic zone, and its amount did not decrease after light reduction. It could be contributed by a *Chloroflexus* sp., which like *C. aurantiacus* is capable of both phototrophic and dark aerobic respiratory metabolism. Alternatively, it could be contributed by a *Thermomicrobium* sp., phylogenetically related to *Chloroflexus* spp. and also capable of aerobic respiratory metabolism in darkness. During the day, oxygen is known to penetrate throughout the layers containing these SSU rRNAs (18). Clearly, prediction of phenotype from environmental perturbation experiments coupled with phylogenetically directed probes can be complicated by metabolic variability.

The vertical distribution of total SSU rRNA, like the vertical distributions of pigment (2; also this study) and lipid biomarkers (35), is consistent with the idea that there is a decrease in the populations of not only phototrophic species but all species with depth. This presumably parallels decomposition of phototrophs and the movement of energy to higher trophic levels with depth in the mat.

The fact that changes in SSU rRNA levels occurred within 1 week, either naturally or in response to an environmental change, suggests that there is a potential for relatively rapid alteration in population dynamics. It should be remembered, however, that ribosome abundance (and hence SSU rRNA abundance) is a function both of the number of individuals in a population of a particular species and of growth rate (7, 27). Thus, the degree to which such changes relate to population dynamics or to physiological adaptation cannot yet be discerned.

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