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Light-Induced Motility of Thermophilic *Synechococcus* Isolates from Octopus Spring, Yellowstone National Park

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This study demonstrates light-induced motility of two thermophilic *Synechococcus* isolates that are morphologically similar but that belong to different cyanobacterial lineages. Both isolates migrated away from densely inoculated streaks to form fingerlike projections extending toward or away from the light source, depending on the light intensity. However, the two isolates seemed to prefer widely different light conditions. The behavior of each isolate was controlled by several factors, including temperature, preacclimation of inocula, acclimation during the experiment, and strain-specific genetic preferences for different light conditions (adaptation). Time-lapse microscopy confirmed that these projections were formed by actively gliding cells and were not simply the outcome of directional cell division. The observed motility rates of individual cells of 0.1 to 0.3 μm s^{-1} agreed well with the distance traversed by the projections, 0.3 to 0.5 mm h^{-1}, suggesting that most cells in each projection are travelling in the same direction. The finding of motility among two phylogenetically unaffiliated unicellular cyanobacteria suggests that this trait may be widespread among this group. If so, this would have important implications for experiments on colonization, succession, diel positioning, and photosynthetic activity in hot spring mats dominated by *Synechococcus*-like cyanobacteria.

Photosynthetic organisms must contend with light that changes over hourly, diel, and seasonal time scales. In the case of photosynthetic microorganisms, slow changes can be dealt with effectively by their propensity for rapid reproduction, i.e., recolonization and regrowth. Short periods of light deprivation may be survived by the lowering of metabolic process rates and utilization of energy reserves until more favorable conditions return. Changes on an intermediate time scale may, however, favor motile populations that can actively migrate into or maintain themselves within favorable conditions. Light-dependent movement of gliding filamentous cyanobacteria is a well-known phenomenon that has been studied for more than 100 years (13). Most strains can move with speeds of one to several micrometers per second. This enables them to perform diel migrations in search of optimal light and nutrient conditions (2, 7, 20). The following concepts have been recommended by an ad hoc committee on behavioral terminology (9): phototaxis, when the direction of movement is regulated by the orientation of the incident light field; photokinesis, when the steady-state speed of movement is determined by the total light intensity; and photophobic response, when the movement is altered (usually by reversing the direction of movement) by a spatial or temporal change in light intensity. Most motile photosynthetic organisms display one or more of these behaviors in their search for suitable light conditions (7). However, additional response patterns for gliding bacteria which do not fall into one of the classes described above have recently been described (20), such as changing (i) the length of inactive versus motile periods, (ii) the frequency of reversing the direction in which a filament is moving, or (iii) the straightness of the traversed path (as opposed to curling up in one place). A combination of all of these responses enables filamentous cyanobacteria such as *Microcoleus chthonoplastes* to position itself in a microbial mat without the aid of a directional light field (20).

While gliding motility is widespread, perhaps universal among filamentous cyanobacteria, the majority of unicellular cyanobacteria appear to be immotile (23), and permanent immotility was considered the general rule among members of the *Synechococcus* group (21). *Bergey's Manual of Systematic Bacteriology* (26) thus lists only 1 strain (PCC6910) of 43 strains as being motile by gliding (some members of marine cluster A are able to swim). However, both R. W. Castenholz (5) and T. D. Brock (3) have observed that some of the thermophilic rod-shaped *Synechococcus* strains were motile and had a gliding motility characterized by pivoting movements and frequent reversals. The apparent motility was never quantified or studied in any detail, presumably because adequate methods were not available.

The morphological simplicity of thermophilic *Synechococcus* strains has led to the assumption that a single species, represented as *Synechococcus lividus*, exists in hot spring microbial mats of western North America (6). All available *S. lividus* isolates from culture collections (i.e., PCC 6715, PCC 6716, PCC 6717, and *S. lividus* type strain Y7CS) showed identical partial 16S rRNA sequences (12). However, investigations with molecular techniques have revealed a remarkable phylogenetic diversity among unicellular cyanobacteria within a single microbial mat. This diversity was apparently concealed by the morphological similarity of the predominating *Synechococcus* spp. within this habitat (10). With serial dilution of inocula to obtain numerically relevant cyanobacterial isolates from this environment, it was possible to solve the apparent discrepancy between previous culture studies and the novel molecular data (12, 24). The *S. lividus* strain found in culture collections appears to be strongly selected for in laboratory enrichment cultures to the exclusion of other *Synechococcus* species from the natural habitat (12).

In this study, we compare the phototactic responses of two of the isolates obtained from the above study. One isolate, C1, was obtained with an undiluted inoculum, and its 16S rRNA shows 99.6% sequence similarity to the culture collection

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strains of *S. lividus*. The other isolate, C9, was isolated after extensive dilution of the inoculum and represents a phylogenetically distinct and hitherto uncharacterized *Synechococcus* species (12). The two strains exhibit only 71.8% similarity in a partial 16S rRNA sequence analysis (12). The phototactic responses of the two isolates were complex and markedly different, indicating that they preferred different light intensities. The gross cell movements were clearly parallel to the incident light field (i.e., phototaxis), and the observed speed was related to light intensity (i.e., photokinesis), but only the latter relationship was shown for individual cells. The observed directionality could be a product of a biased random walk, and we hesitate to use the term "phototaxis" until a direct orientation of individual cells due to a directional light field has been demonstrated. We therefore used the unspecific term "light-induced motility" in the title. For both strains investigated, the speeds of movement were relatively low ($\leq$0.3 μm/s), especially at room temperature ($\leq$0.05 μm/s), which probably is at least part of the reason why most thermophilic *Synechococcus* strains have hitherto been considered immotile. We have thus used digital image analysis as a convenient tool with which to analyze these very slow and complex motility patterns.

MATERIALS AND METHODS

Organisms and growth conditions. The two isolates investigated, C1 and C9, were obtained from a 50°C site in the shoulder region of Octopus Spring, Yellowstone National Park, as previously described (12). C1 was isolated from a 50-ml enrichment culture inoculated with 2.5 cm^3 of surface mat material containing approximately 2.5×10^{10} *Synechococcus* cells, whereas the enrichment from which C9 was isolated was prepared by serial dilution so that the effective inoculum was reduced to 2.5×10^{-8} cm^3 of mat material, corresponding to approximately 250 *Synechococcus* cells. C1 is thus likely to be the *Synechococcus* isolate best adapted to the laboratory conditions, whereas C9 could be a numerically more important member of the natural community (12). Both isolates form 8-μm-long, 2-μm-wide slightly curved rods of relatively uniform appearance. The isolates were grown on 0.75% agarose slants containing medium DG as previously described (6) at 50°C and were illuminated by approximately 35 microeinsteins m^{-2} s^{-1} from fluorescent bulbs.

Plate incubations. Disposable petri plates with medium DG containing 0.75% agarose (SeaKem; FMC, Rockland, Maine) were incubated at 50°C and illuminated by directional light from two GroLux fluorescent bulbs at three different light intensities of 0, 4, and 35 microeinsteins m^{-2} s^{-1} by placement of the petri plates inside different vessels in the incubator. Full sunlight at the sampling site in Yellowstone National Park is approximately 1,800 microeinsteins m^{-2} s^{-1}. Half of each plate was covered with black tape to ensure a directional light field by elimination of reflected light. Each plate was inoculated with a dense 1-mm-wide linear streak of cyanobacteria perpendicular to the direction of the incident light at the boundary between the eclipsed half of the plate and the transparent half. The development of fingerlike projections during a 4-day incubation was monitored at regular intervals by temporarily removing the plates from the incubator, placing them on an overhead projector, and tracing the magnified outline of the projections on a wall.

Temperature-controlled microscope stage. A heated microscope stage was constructed by connecting a modified coffee mug warmer to a rheostat and placing it on the microscope stage. Only the heating element of the mug warmer was used after holes had been drilled for phase-contrast illumination. The microscope slides and petri plates could thus be kept at different temperatures (±2°C) for extended periods of time. However, precipitation of water vapor on microscope lenses at higher incubation temperatures was a problem, especially when high-magnification objectives with a short working distance were used. The killed control experiments were done by observation of a motile cell cluster on an agar plate before and after application of a 4% formaldehyde or 15% peroxide droplet.

Agar well slides. To solve the precipitation problem, special agar-coated slides were made. Ordinary microscope slides were covered with a 2-mm-thick layer of 0.75% agarose in medium D (6). A small plastic square (7 by 7 mm, 0.5 mm thick) cut out from the lid of a petri dish was embedded in the agar and removed after solidification, thus creating a small well with an agar bottom in which the cyanobacteria were placed. The well was then filled with medium D and covered with a large coverslip. The agar surrounding the observation area prevented rapid desiccation during observations at elevated temperatures, and the coverslip prevented water vapor from reaching the objective. The slides were illuminated with a dissection microscope lamp placed as close to the slide as possible to achieve the highest possible light intensity (i.e., 90 to 160 microeinsteins m^{-2} s^{-1}).

Microscopy and image acquisition. An Axioskop microscope (Zeiss, Oberkochen, Germany) with ×2.5, ×10, and ×40 plan Neofluar objectives equipped with a Zeiss video camera was used to obtain phase-contrast images at different magnifications. Images were acquired with the built-in frame grabber of a PowerMac 7500 (Apple, Cupertino, Calif.) using the green video line from the camera. Average images of four frames were taken at defined time intervals that were scaled to give maximum contrast, combined into short movie segments, and stored digitally for further processing.

Image manipulations and analysis. Image acquisition, difference imaging, cell tracking, and all subsequent analysis were done with the program NIH Image, version 1.59. NIH Image was written by Wayne Rasband, National Institutes of Health, Washington, D.C. The program is freely available from the anonymous FTP site zippy.nimh.nih.gov on the Internet. Custom-made subroutines for image acquisition and cell tracking are available on request from the corresponding author. The composite image in Fig. 1A was assembled from 27 individual images with the program Adobe Photoshop (Mountain View, Calif.).

RESULTS

Structure of fingerlike projections extending toward a light source. When a plate of agar medium inoculated with a thin line of C1 cells was illuminated with a perpendicular light field of moderate intensity (i.e., 35 microeinsteins m^{-2} s^{-1}), the inoculated cells started to move and form protrusions. A typical picture of the resulting fingerlike outgrowths after 50 h of incubation at 50°C is shown in Fig. 1. The similarity to the fingerlike projections formed by another *Synechococcus* strain, PCC 6910, is remarkable (shown in Fig. 12 of reference 23). The individual fingers pointing towards the light source all have about the same width, 180 to 300 μm (i.e., less than 100 cells wide), and run remarkably straight toward the light source. A prominent feature was the "patchy" appearance, with clearly delineated areas having a distinctly darker shade (green in color). Higher magnification revealed that these regions correspond to areas in which more than one layer of cells was found atop another (i.e., bilayers, or in very dark areas, trilayers, of cells). These multilayered areas were primarily observed toward the tips of the projections (see enlargement in Fig. 1D). Most areas of the structures were, however, of a discrete uniform light green color that corresponded to only a single layer of densely packed cells.

Projections away from the light. Initial projections that formed soon after inoculation with C1 moved away from the light. The leading cells in these projections apparently continued to move toward the dark. However, few other cells continued to move in that direction, and small clumps of cells soon separated from the bulk part of the inoculum, leaving a faint trail behind (see projections on the left side of Fig. 1A, one of which is magnified in Fig. 1B). The trail is visible by phase-contrast microscopy after a slight defocusing (Fig. 1C). The trail protrudes from the surface because it can easily be scraped away with an inoculation loop. The empty trail is not visible under water cover (probably due to a refractive index similar to that of water); however, if the water is removed, the trails reappear at the exact same location. We thus assume that the trails contain exopolymers, since compounds with a low molecular weight would diffuse away rapidly under the water cover and changes in surface tension should be eliminated by wetting and drying.

The empty slime tracks (often more than 1 mm long) found in the projections extending away from the light source (Fig. 1A and B) provide convincing macroscale evidence that the cells are actively moving and not only dividing in a preferred direction during growth. This was further confirmed by time-lapse microscopy (see below). The empty tracks were observed even after relatively short incubation periods (<5 h) which appear to be too short for substantial cell decay, and the tracks were completely transparent without any evidence of cell debris.

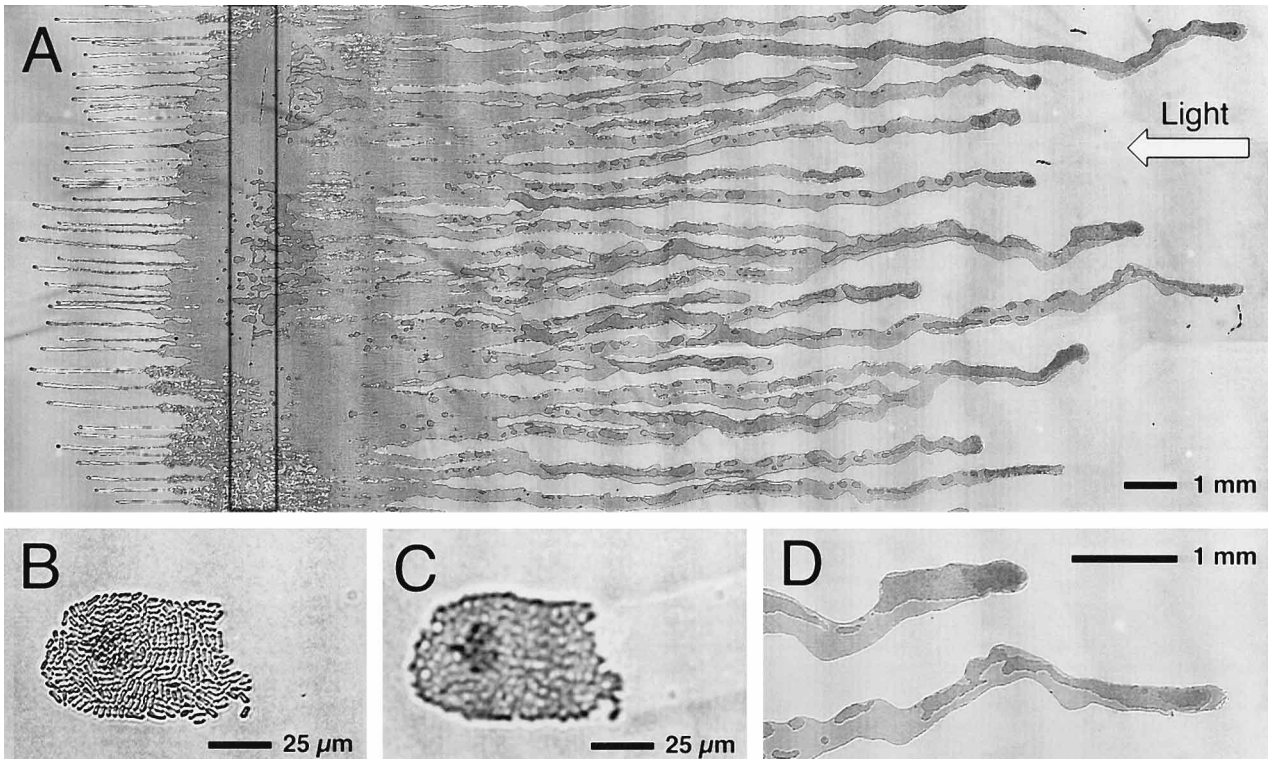

FIG. 1. Fingerlike projections formed perpendicular to the line of inoculation after illumination of a linear streak of *Synechococcus* sp. isolate C1 with 35 microeinsteins m^{-2} s^{-1} for 50 h at 50°C. (A) Collage of 27 digital images combined to show the full length of the projections. An approximate boundary of the original inoculum is delineated by the black rectangle. The white arrow indicates the direction of the incident light. (B) Monolayered cluster of cells at the tip of the deserted projections extending toward the dark side of the plate (see Results). (C) Defocused image that allows the slime track left by the moving cell cluster in panel B to be visualized. (D) Enlarged view of the patchy structure of the tips of the projections extending toward the light source. Darker patches are formed by multiple layers of cells gliding atop each other.

Isolate-specific differences in motility under various light conditions. The temporal development of the fingerlike projections associated with each isolate at three different light intensities is shown in Fig. 2. Panels A to C show the length of projections made by isolate C1 when incubated at different light intensities as a function of incubation time. Panels D to F depict the outcome of the equivalent experiment with isolate C9. The C9 projections extending away from the light source remained filled with cells, as opposed to the empty slime tracks left by C1 cells moving toward the dark side of the plate (described above). Isolate C1 was generally quite motile, and even when incubated in complete darkness, the cells slowly started to spread out over the plate (Fig. 2A). This basic motility is strongly enhanced at elevated light intensities. At a relatively low light intensity of 4 microeinsteins m^{-2} s^{-1}, the cells do not show directional motility (i.e., projections toward and away from the light source are of equal length [Fig. 2B]). Only at a higher light intensity of around 35 microeinsteins m^{-2} s^{-1} are the cells able to move directionally toward the light source (Fig. 2C). The preferred light intensity of this isolate remains unknown, but it is evidently equal to or greater than 35 microeinsteins m^{-2} s^{-1}.

Isolate C9 remains immotile when incubated in the dark (Fig. 2D). It shows a slow but clearly directional movement toward the light at a low light intensity of 4 microeinsteins m^{-2} s^{-1}, (Fig. 2E), whereas it moves quite rapidly away from the light when incubated at 35 microeinsteins m^{-2} s^{-1} (Fig. 2F). This isolate evidently prefers a light intensity of between 4 and 35 microeinsteins m^{-2} s^{-1}, most likely closer to the former value than to the latter. The preferred light intensity with respect to motility, which presumably corresponds to the optimal light intensity for growth, differed widely between the two isolates. However, the maximum increases of projection length were quite similar for both isolates (compare the initial movement of cells toward the dark side in Fig. 2C and F). Peak motility rates of about 0.3 to 0.5 mm h^{-1} were measured for both isolates during the initial formation of projections toward the dark side (i.e., the slopes in Fig. 2C and F). This rate corresponds to about 0.1 μm s^{-1}. For both isolates, the highest motility was realized under illumination with the highest experimental light intensity, so it is conceivable that even higher rates of movement could be achieved at even higher light intensities.

Complex behavior. The photic behavior of these isolates cannot be explained by constant positive or negative phototaxis, because the temporal development of the projections appeared to be composed of several distinct phases. The initial movement of C1 was thus only away from the light when incubated at 35 microeinsteins m^{-2} s^{-1} (Fig. 2C), with projections toward the shaded side eventually extending several millimeters. Only after a 10-h lag phase did projections develop toward the light, and they then continued to grow at a fairly constant rate throughout the remainder of the experiment. The experiment was repeated several times with identical outcomes. The two isolates used for the experiment depicted in Fig. 2 were preacclimated under identical conditions (17 microeinsteins m^{-2} s^{-1}), so the observed differences in behavior are ascribed to different genetic adaptations of the two isolates. The effect of acclimation was investigated for strain C1 by observation of the motility at 35 microeinsteins m^{-2} s^{-1} after 72 h of preac-

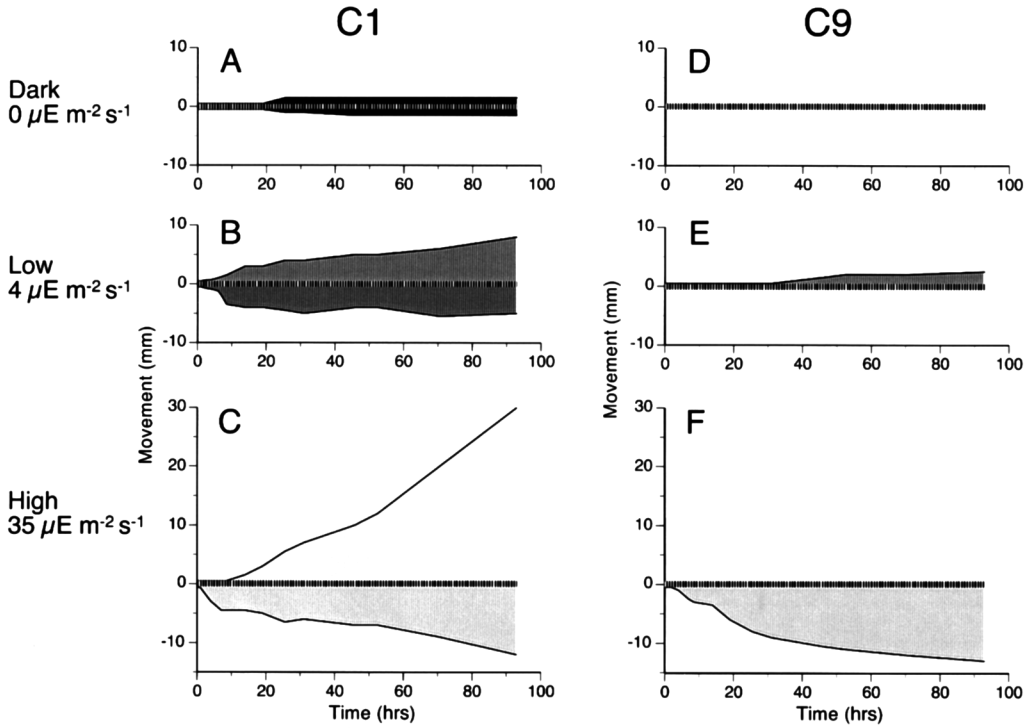

FIG. 2. Chronological development of fingerlike projections for two different *Synechococcus* sp. isolates: isolate C1 (A to C) and isolate C9 (D to F). The successive lengths of the projections are depicted for when cells were incubated in the absence of light (A and D), at low light intensity (4 microeinsteins [μE] m^{-2} s^{-1}) (B and E), and finally at high light intensity (35 microeinsteins m^{-2} s^{-1}) (C and F). Movement toward the light is indicated by positive values, whereas movement away from the light source is indicated by negative values. Both inocula were preacclimated by growth on agar slants at the same light intensity of approximately 17 microeinsteins m^{-2} s^{-1}. The hatched bar along the abscissa represents the width (≈1 mm) of the inoculum.

climation at three different light intensities (approximately 0, 4, and 30 microeinsteins m^{-2} s^{-1}, respectively). Most cells in an inoculum acclimatized to 30 microeinsteins m^{-2} s^{-1} moved toward the light source in a pattern similar to the one shown in Fig. 1A (but still with a 10-h lag phase), whereas most cells in an inoculum acclimatized to 4 microeinsteins m^{-2} s^{-1} moved away from the light source and continued in this direction for several days. The cells preacclimatized in darkness appeared unhealthy and remained immotile for the first 30 h, after which they slowly started to spread over the plate in both directions.

Motility of individual cells. We have previously used differential imaging (i.e., subtraction of images taken at different time points to visualize moving cells) to investigate the motility of *Microcoleus* sp. cells under different light conditions (20). A similar approach was used to investigate movement of *Synechococcus* isolates. The difference image between two succeeding frames, Fig. 3A and B, taken 20 s apart is shown in Fig. 3C. The position of two cells moving away from each other (indicated by a and b in the first two frames) is highlighted. Theoretically, tracks of moving cells can be seen in the difference image as black paths in the direction in which the cells are moving, leaving white paths behind. The resulting paths in the difference image for the movement of cells a and b are indicated by black and white arrows in Fig. 3C. The total amount of movement can thus often be quantified by counting black or white pixels in the difference image. This procedure works well for rapidly moving filamentous bacteria (20); however, the dense packing of *Synechococcus* cells in these clusters (possibly constrained by the extent of the excreted exopolymers) generates more complex movements as cells collide with each other and push each other around. The resulting difference image produced by the complex and nonlinear movements of individual cells in the dense cell clusters is consequently difficult to interpret.

Killed control experiments. To verify that the complex motions were not merely slow Brownian motions or convective currents in a viscous exopolymer medium, we applied a droplet of 4% formaldehyde to a cluster of motile cells and acquired a second set of images of the same cell cluster (Fig. 3D and E). It is evident from the difference image (Fig. 3F), as well from visual impressions of a speeded-up playback of the movie, that all of the cells were now completely immotile. A potential problem with killing the cells with formaldehyde is a possible molecular cross-linking of the exopolymer matrix (i.e., increasing the viscosity and thus reducing Brownian motion). However, a similar treatment with 15% peroxide had an identical effect. The large difference between the motility of live cells and killed controls thus provides compelling microscale evidence that individual cells are actively moving (i.e., the difference between Fig. 3C and F). The slow movement of *Synechococcus* sp. cells even at elevated temperatures necessitates long time intervals between exposures which require a high physical stability of the microscope stage, because even the slightest translation will produce a shadow image on the difference picture. The difference image in Fig. 3F shows a faint extruded outline of the cells, which is not due to individual cell motility but instead is due to the instability of the microscope stage employed in the study. This problem also restricts the use of differential imaging in measuring total cell movements, because even a small frameshift by <1 pixel would, to the image analysis algorithms, be indistinguishable from real movement.

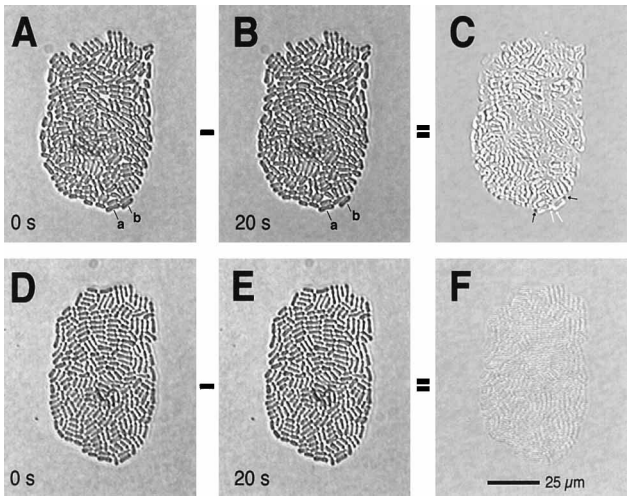

FIG. 3. Assessment of motility of C1 in projections formed on agar plates by difference imaging. Comparison of digital images acquired at 20-s time intervals by subtraction of one from the other reveals subtle motions (<0.05 μm/s) which are otherwise not noticeable. (A and B) Consecutive images of live cells incubated at 25°C without a directional light field. (C) Resulting difference image that reveals an irregular distribution of light and dark areas resulting from random movement of the individual cells. (D and E) Consecutive images of the same cell cluster under the same conditions after a killing treatment with 4% formaldehyde. The very faint difference image (F) resembles the two originals and is due to a slight general displacement of the cell cluster during the experiment. Movements of the leading and trailing ends of two cells (a and b) are indicated by dark and light arrows, respectively. The scale bar in panel F applies to all images.

Tracking single cells. Recording the movement of individual motile cells manually is clearly much more time-consuming than bulk analysis of difference images (although less so when aided by suitable image analysis subroutines), but the results appear to be more accurate and are less prone to error due to the problems described above. We thus used single-cell tracking of motile C1 cells to quantify the effect of different environmental conditions such as light, temperature, bactericides, and water cover. C1 was used in this study because it was generally more motile and easy to observe than C9. Ten of the most motile cells were selected in each movie acquired under a particular set of conditions. The individual cells were monitored for 20 consecutive frames under each set of conditions, and their individual overall movement was calculated. All measurements without liquid cover were made on agar plates with low magnification (×100 or ×160), whereas most of the measurements with liquid cover were made on agar well slides (see Materials and Methods) at ×400 or ×640 magnification. Comparisons showed that agar plates and agar well slides gave equivalent results (data not shown). However, the larger magnification possible with agar well slides enhanced the accuracy of the motility estimates. An example of tracking of a single cell in four consecutive frames is shown in Fig. 4, whereas the average movements of all 10 cells under various conditions are summarized in Fig. 5. A total of 20 movies obtained under different conditions were analyzed, but only 8 representative examples, showing the general trends, are included in Fig. 5. It should be noted that only motile cells were recorded, which is problematic when very few cells are motile, such as after bactericidal treatments. The very small movement registered after formaldehyde and peroxide treatment (Fig. 5, columns 6 and 8, respectively), thus merely reflects the instability of the microscope stage, as explained above, and possibly Brownian motion. It should also be noted that selection for motile cells only reveals photokinesis (i.e., changes in the speed of moving cells). We could not by such measurements detect changes in frequency of movement or in frequency of reversal of movement direction, both of which were found to play a very important role in the light-dependent motility of *Microcoleus* sp. (20).

(i) Temperature effects. The results of a representative set of experiments designed to demonstrate the combined effects of key environmental parameters on the motility of *Synechococcus* sp. strain C1 are shown in Fig. 5. Temperature had the most pronounced effect on the motility of individual cells in that the motility rate appeared to be halved when the temperature was reduced from 45°C to 35°C (compare Fig. 4, columns 1 and 3) and was halved again when the temperature was further reduced from 35°C to 25°C (compare Fig. 4, columns 5 and 7). At 45°C, the average motility was 0.27 ± 0.02 μm/s (Fig. 5, column 1), which is barely visible to the patient observer under a high magnification. However, at 25°C without directional illumination, the motility was reduced to 0.05 ± 0.01 μm/s (Fig. 5, column 7), which is too slow to be notable even at high magnification unless recorded by a camera and played back at a faster rate.

(ii) Light effects. The presence of a directional light field also appeared to influence the motility of *Synechococcus* sp. strain C1. Incubation in darkness reduced the motility to about half of the original value at 45°C (Fig. 5, columns 1 and 2), whereas the effect was substantially lower at 35°C (Fig. 5, columns 4 and 5). It should be noted, however, that the applied time interval between experiments (approximately 10 min) might not have been long enough to permit adjustment to the new conditions. We have not investigated the effect of different light intensities, and this is indeed a complex problem, because gradual light acclimation or adaptation is likely to occur over different time scales, as is evidenced by the complex motility behavior in the plate incubation experiments.

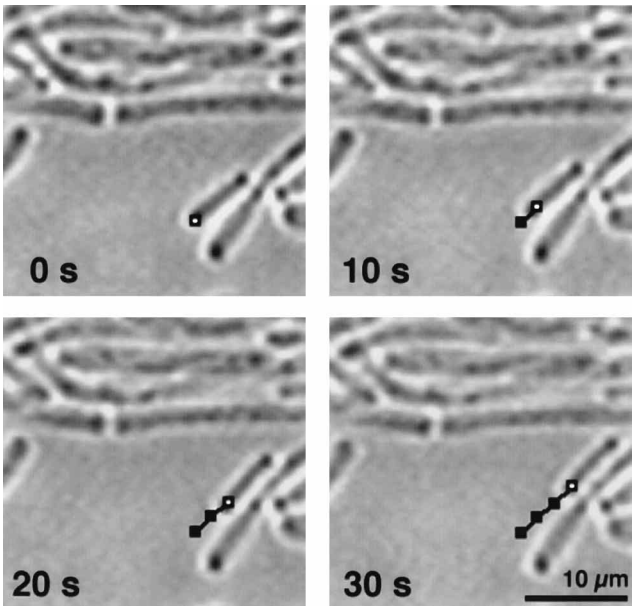

FIG. 4. Tracking of a single *Synechococcus* sp. isolate C1 cell. (A to D) Identical view areas in four consecutive frames acquired at 10-s intervals. The track of a single cell is indicated in black. White diamonds indicate the current position of trailing end of cell, while black diamonds indicate the position in previous frames. The scale bar in the last frame applies to all frames.

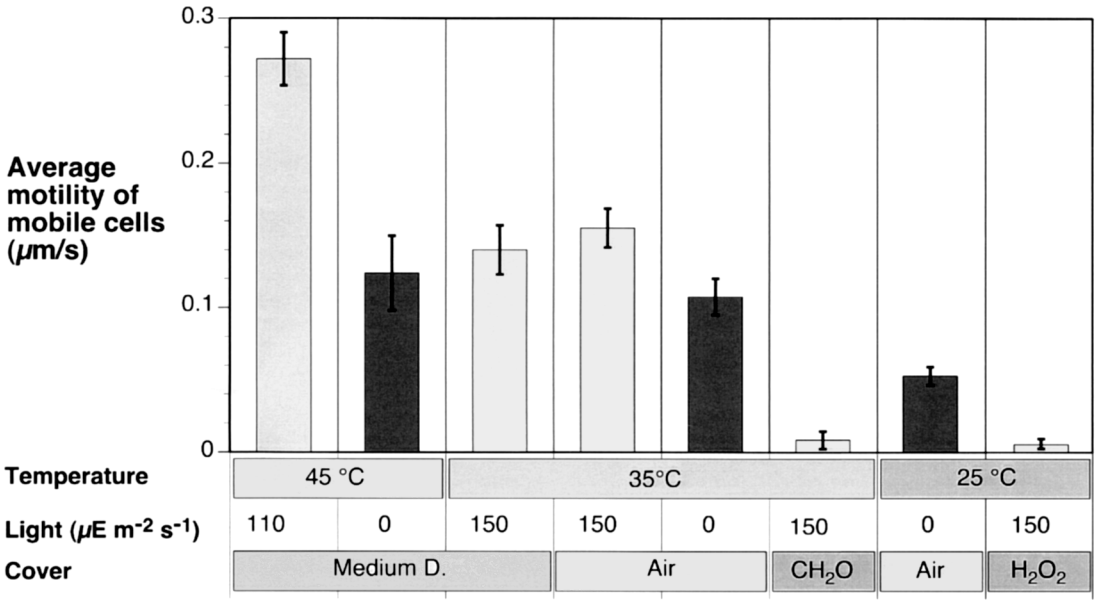

FIG. 5. Average motility of mobile *Synechococcus* sp. isolate C1 cells under different conditions. Ten individual cells were tracked in a series of movies acquired under different conditions, and the average motility under each set of conditions is represented by the height of the bars. The error bars represent 95% confidence limits of the mean. The table beneath the columns indicates the conditions used in each experiment. Cover, type of medium enclosing the cells; Air, atmospheric air (i.e., no liquid phase covering the cells); CH_2O, 4% formaldehyde in medium D; H_2O_2, 15% peroxide in distilled water. Only mobile cells were examined, and the faint motility of the formaldehyde- or peroxide-killed samples was most likely due to Brownian motion and instability of the microscope stage (see text). μE, microeinsteins.

(iii) Water cover effects. We tested the relative motility directly on air-exposed or medium-covered agar plates because it seemed that the type of cover might be important (e.g., by dissolving or desiccating the exopolymer layer or by changing the surface tension). However, no significant changes were observed (Fig. 5, columns 3 and 4), possibly because the cells glide within an exopolymer matrix that is relatively unaffected by exterior conditions. Adaptations which enable the cell to glide efficiently through a slime matrix seem highly advantageous for these cells which normally exist in a microbial mat environment. Similar adaptations have been found for filamentous cyanobacteria (7).

DISCUSSION

Our experiments establish that *Synechococcus* sp. strains C1 and C9 are motile organisms and are thus capable of actively positioning themselves within favorable environmental conditions. The empty slime tracks produced shortly after inoculation provide macroscale evidence for motility, because such empty tracks are unlikely to be formed by growth alone. The difference between the observed microscale movements of live cells and the lack of movement of killed controls provides further evidence that cannot be explained by convective currents or complex Brownian motion.

It is interesting that the motility of the most rapidly moving individual cell (0.1 to 0.3 μm/s) matches fairly well the development rate of the fingerlike projections (0.4 mm/h ≈ 0.1 μm/s). The cells in the tips of these projections must therefore travel in the correct direction in a relatively concerted fashion, which seems more efficient than what would be expected from a common "biased random walk" mechanism. The motility of isolate C1 is slow (<0.3 μm/s) compared to that reported for most filamentous gliding bacteria (usually >1 μm/s) (1, 8). The motility of C1 is further reduced at room temperature (≈0.05 μm/s). Strain C9 appears to be relatively immotile unless the light conditions are appropriate. Given the experimental difficulties, it is not surprising that the motility of thermophilic *Synechococcus* has not been quantified before. Video imaging and accelerated playback and/or image analyses, such as differential imaging, are generally required to detect such slow motility at the individual cell level.

The rapid light-induced movements of filamentous thermophilic *Oscillatoria* sp. and *Phormidium* sp. in hot spring microbial mat communities are well documented (4–7). Slow gliding motilities are less frequently studied, but a phototactic movement of 0.03 μm/s at 22°C has been described for some unicellular baeocytes of the order "*Pleurocapsales*" (27). The twitching movements of some thermophilic *Synechococcus* strains have been mentioned (3, 5), but no quantification has, to our knowledge, been attempted.

The complex behavior observed for C1 under high light intensities could be explained as an acclimation to different light intensities, possibly due to a readjustment of the photosystems to take advantage of changing light conditions. The initial movement toward the dark created a densely filled projection, followed by an apparent readjustment and a subsequent movement toward the light, leaving empty slime tracks with only a tiny cluster of cells at the tip. It seems likely that the initial inoculum consisted of more than one population acclimated to different light intensities. For example, cells at the more shaded bottom of the dense growth on the agar slants used for inoculation might be acclimated to lower light intensity than cells at the surface. Upon transfer to the higher light intensity of the plate, they might initially move away from the light until their photosynthetic machinery could be adjusted to cope with the higher light intensity.

Clearly the ability to position themselves favorably by virtue of their motility can give these *Synechococcus* isolates an ecological advantage in a changing environment with steep fluctuating gradients in light, nutrients, and other physical and chemical parameters. However, a prerequisite must be that the

cost of movement is not too high (in agreement with previous estimates for gliding cyanobacteria [7]) and that the bacteria live in a gradient system in which physical and/or chemical parameters change within a relatively small spatial scale. With regard to the cost of movement, novel investigations of the Octopus Spring microbial mat system have revealed that relatively little of the CO_2 fixed by photosynthesis is used for new growth (19). Most is stored as polysaccharides and apparently fermented in the absence of light when the mat becomes anoxic. However, it is possible that some of these polysaccharides are used as an energy source for motility or are excreted as part of the gliding mechanism.

The observed motility was highly temperature dependent, as has been shown in other gliding cyanobacteria (5). This could be due to a high-temperature optimum for the enzymatic apparatus responsible for motility or to a decreased cellular metabolism, implying that fewer resources are available for motility at low temperature. Indirect causes such as an increased viscosity (or stickiness) of the surrounding exopolymers may also be involved. Nonetheless, because these organisms presumably prefer high temperatures, it would be preferential for them to minimize their motility under favorable conditions and to increase their motility if the temperature conditions are suboptimal. The indication that this was not the case might suggest that they do not usually use their motility to position themselves in a thermal gradient. This agrees well with the observation that thermal gradients in Octopus Spring occur over meter scales which would be impossible for cells to traverse anyway. However, recent investigations with optical microsensors have revealed that the light field changes dramatically within the upper 0.5 to 2 mm of a microbial mat (2, 14–18). These distances can easily be traversed by *Synechococcus* cells within a few hours at the observed motility rates (0.4 to 1 mm/h). It is thus not surprising that the cells do appear to respond to a directional light field by moving toward or away from the light source, depending on the light intensity (Fig. 2).

The motility rate detected in this study is sufficient to enable the cyanobacteria to move vertically through the photic zone (<2 mm) on a diel basis, as has been observed in other environments (2). The possibility of a recurring diel migration, which would likely be different for populations adapted to different light intensities, has profound implications for the interpretation of the results obtained from experiments conducted with microbial mat environments constructed by *Synechococcus*-like cyanobacteria. It is possible, for instance, that a very-low-light-adapted strain such as C9 could move to the top of the mat during the night, but down to more favorable light conditions during the middle of the day. High-light-adapted strains such as C1 could move in the opposite direction. This would imply that different strains are positioned near the top of the mat at different time points. Short incubations to measure CO_2 fixation and/or metabolism of different tracers added to the overlying water would thus measure the activity of different motile populations occupying different spatial positions at different times of the day; changes in metabolic rates could simply reflect different populations moving into or out of the surface layer.

Synechococcus motility may also have implications for our understanding of colonization events such as those occurring after physical disturbances to the mat environments (11). Recolonization might involve not only random settling of inocula from upstream sources which slowly spread over the surface due to their high reproductive rate but also active movement of cells from adjacent areas. This might provide an explanation for the slow upstream colonization of effluent channels in hot springs in which the temperature has slowly declined to levels that permit existence of cyanobacteria (e.g., during a cold winter period) but in which there is clearly no upstream mat to provide a passive inoculum.

This study demonstrates the existence of genetically distinct strains that are apparently adapted to different light intensities with respect to photomovement and thus complements the set of temperature-adapted strains that has been found previously (6, 7, 25). Previous hybridization experiments utilizing oligonucleotide probes targeting uncultivated cyanobacterial populations inhabiting the Octopus Spring mat revealed different responses to reduced light intensity, which were interpreted as reflecting different light preferences (22). A vertical stratification of genetically distinct *Synechococcus* populations has recently been observed in an adjacent spring, Mushroom Spring, Yellowstone Park (unpublished results). How prevalent motility is among the thermophilic *Synechococcus* species is still unresolved. We have not yet investigated any of the well-characterized strains from culture collections, but it should be noted that C1 and C9 are only very distantly related and thus that motility is a phylogenetically widespread trait even among the unicellular cyanobacteria. We have recently obtained additional isolates (by a similar serial-dilution-of-inoculum approach) from Octopus Spring (24) that show considerable motility, so the phenomenon might be more widespread than previously recognized.

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