A Cyanobacterium Capable of Swimming Motility

Abstract. *A novel cyanobacterium capable of swimming motility was isolated in pure culture from several locations in the Atlantic Ocean. It is a small unicellular form, assignable to the genus* Synechococcus, *that is capable of swimming through liquids at speeds of 25 micrometers per second. Light microscopy revealed that the motile cells display many features characteristic of bacterial flagellar motility. However, electron microscopy failed to reveal flagella and shearing did not arrest motility, indicating that the cyanobacterium may be propelled by a novel mechanism.*

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The cyanobacteria (blue-green algae) are morphologically and developmentally one of the most diverse groups of prokaryotes, ranging from simple unicellular forms to complex filamentous organisms, many of which display gliding motility during all or part of their developmental cycles. Gliding motility is expressed only when cells are in contact with a solid surface, and may reach speeds of several micrometers per second in some of the larger filamentous forms (*1*). The mechanism responsible for gliding motility remains one of the major unsolved problems in prokaryotic cell biology. Notably, visible external organelles have not been associated with this form of movement (*1*). In contrast, "swimming" (movement through a homogeneous liquid medium) generated by flagella is widespread among other groups of prokaryotes (*2*).

We report here the isolation and characterization of a cyanobacterium capable of swimming motility. To our knowledge, this is the first substantiated report of swimming motility in cyanobacteria (*3*). The swimming strains appear to lack bacterial flagella or other visible external organelles of motility, even though their swimming behavior has many of the features of flagellar motility when examined by light microscopy.

The swimming strains are marine unicellular forms belonging to the genus *Synechococcus* sensu Rippka (*4*). Members of this genus have been described that are capable of surface-associated irregular gliding at speeds of a few micrometers per minute (*1*, *5*). This property is rare, occurring in only 1 of 28 strains of *Synechococcus* in the Pasteur Institute culture collection (PCC 6910) (*6*). Representatives of the genus occur at high concentrations (*7*) and contribute significantly to primary biomass production (*8*) in the world's temperate and tropical oceans. As part of a study to determine the role of these cyanobacteria in the marine environment, we have isolated more than 40 closely related strains of marine *Synechococcus*.

Six strains of *Synechococcus* capable of swimming motility have been isolated (*9*), five from the Sargasso Sea and one from the temperate south Atlantic Ocean (Table 1). The swimming strains of *Synechococcus* are morphologically and physiologically similar to the many open-ocean nonmotile isolates in the Woods Hole culture collection. The swimming strains have coccoid to rod-shaped cells 0.7 to 0.9 μm in diameter and 1.25 to 2.5 μm in length (Fig. 1A) that divide by binary fission in a single plane. They have a typical synechococcoid ultrastructure (Fig. 1B) that is indistinguishable in thin sections from that of the nonmotile marine isolates of *Synechococcus*. The marine isolates of *Synechococcus*, like all other cyanobacteria, contain chlorophyll a as their primary photosynthetic pigment and phycobiliproteins as accessory light-harvesting pigments. The motile strains contain phycoerythrin as their predominant phycobiliprotein, a feature that characterizes all the open-ocean isolates of *Synechococcus* (*10*).

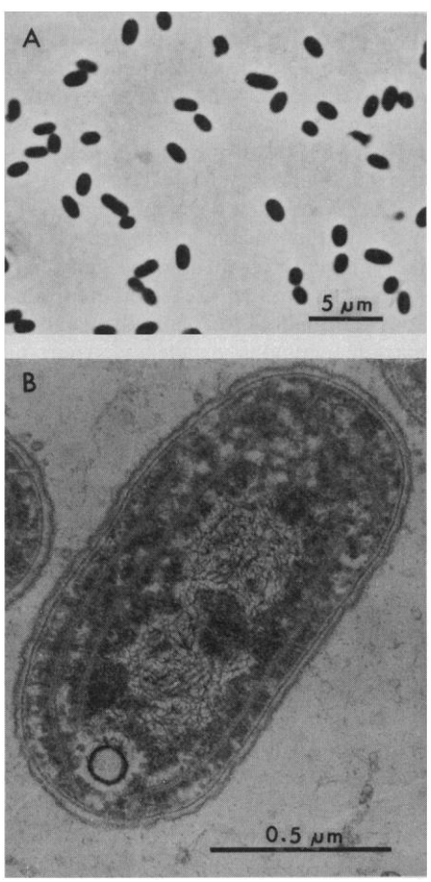

Fig. 1. (A) Phase-contrast photomicrograph of *Synechococcus* strain WH8011. (B) Transmission electron micrograph of a thin section of *Synechococcus* strain WH8112.

The swimming strains share three ecologically important physiological traits with all the open-ocean strains of *Synechococcus* so far examined. They are obligately marine, as evidenced by elevated requirements for Na^+, Cl^-, Mg^{2+}, and Ca^{2+} for growth (*11*). They are obligate photoautotrophs that cannot use sugars or organic acids as sole carbon sources either photoheterotrophically or chemoheterotrophically (*12*). They cannot fix nitrogen in the presence of air, nor can nitrogenase be induced under anaerobic conditions (*13*).

The DNA base ratios of the four axenic swimming strains (Table 1) form a tight cluster in the middle of the range for the entire open-ocean strain cluster, 54 to 62 moles percent guanine plus cytosine (*14*).

Swimming cells in liquid cultures examined during exponential growth by phase contrast or epifluorescence light microscopy had the following properties. More than half of the cells were actively motile, and moved through the medium without touching any surface. Long rod-shaped cells swam in relatively straight paths, whereas more coccoid cells looped and spiraled about, indicating that cell morphology affects directionality (Fig. 2). Individual cells sometimes rotate end over end at 3 to 5 revolutions per second. Occasionally cells attached themselves to the slide or cover slip and pivoted about one pole clockwise or counterclockwise at 0.5 to 1 revolution per second. Attached cells were never observed to glide along a surface.

Swimming speed, measured from video tracings of individual cells at 1/6-second intervals, ranged from 5 to 25 μm/sec. The variation in speed was associated with the age and condition of individual cultures and not with variations in light intensity. Translocation was accompanied by cell rotation, as demonstrated by swimming cells in the process of division in which the two daughter cells were attached at an angle; thus both thrust and torque contributed to their movement. Swimming cells of *Synechococcus* could often be observed

for more than 1 minute without showing interruptions in translocation analogous to the characteristic "tumbles" of flagellated bacteria (*2*). As in other forms of movement in prokaryotes, experiments with a variety of inhibitors indicate that swimming in *Synechococcus* is driven by proton motive force (*15*).

The swimming strains of *Synechococcus* did not show photokinesis or photophobic responses, nor did they demonstrate positive or negative phototaxis in liquid or on semisolid preparations (*16*). Rates of motility were similar for cells incubated with light of different intensities or kept in the dark for up to 12 hours. This suggests that, in the dark, motility may be driven by oxidative metabolism of glycogen reserves in a manner similar to that shown for gliding in *Oscillatoria princeps* (*17*). Lack of a photophobic response by swimming *Synechococcus* is indicated because they did not accumulate in a light trap experiment and they did not reverse direction or alter their swimming pattern in response to a rapid reduction in light intensity (*1*).

The most striking feature of these strains is the apparent absence of flagella or any other organelle that might be associated with motility. Transmission electron microscopy of negatively stained preparations did not reveal flagella, whereas flagella were routinely observed in control bacteria examined by this technique (*18*). Examination of thin sections by TEM did not reveal flagella or unusual features in the outer cell envelope or the periplasmic space (Fig. 1B) (*19*). Preliminary examination by high-intensity dark-field light microscopy, a technique that allows visualization of individual bacterial flagella (*20*), also did not reveal flagella.

Shearing experiments provided further evidence that the swimming strains of *Synechococcus* lack conventional bacterial flagella. Brief periods of blending are sufficient to shear flagella from bacterial cells without affecting cell viability (*21*). We conducted shearing experiments with the motile strains of *Synechococcus* grown in basal medium (*9*) with 8.75 m*M* sodium nitrate. Control bacteria, *Escherichia coli* H102 and *Vibrio parahaemolyticus*, which have unsheathed peritrichous and sheathed polar flagella, respectively, lost motility after 10 to 15 seconds of shearing; whereas swimming in *Synechococcus* was not impaired even after 15 minutes of continuous blending (*22*).

Several properties of *Synechococcus* indicate that the ecological advantage of motility is not associated with responses to light. First, the motile cells lack classical photokinetic and photophobic behavior. Second, a cell swimming constantly in a straight line at 25 μm/sec could cover only 2 m in 24 hours, a distance of almost no consequence with respect to light quantity or quality in the open ocean. Finally, because of their size, *Synechococcus* cells behave like colloidal particles in seawater, which results in their movement being dominated by the physical mixing processes in the water column. It seems more likely that motility enables these cyanobacteria to respond chemotactically to nutrient-enriched micropatches or microaggregates, which are currently believed to be important to nutrient cycling in the euphotic zone (*23*).

Table 1. Isolation data and DNA base composition of swimming strains of *Synechococcus*.

Strain	Isolation data			DNA base ratio (mol % GC)*
	Location	Date	Depth	
		Axenic		
WH8011	34°N, 65°W	June 1980	25 m	59.3
WH8103	28°N, 67°W	March 1981	Surface	58.9
WH8112	36°N, 66°W	October 1981	20 m	59.8
WH8113	36°N, 66°W	October 1981	60 m	60.5
		Nonaxenic		
WH8401	39°S, 49°W	March 1984	10 m	ND†
WH8406	30°N, 77°W	December 1984	50 m	ND

*Moles of quanine plus cytosine per 100 mol of DNA. †ND, not determined.

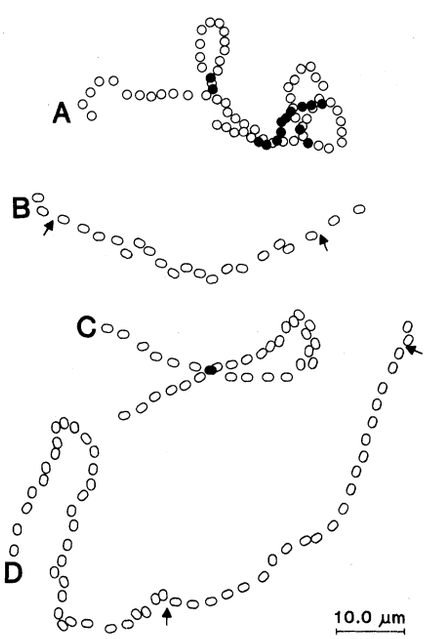

Fig. 2. Tracings of swimming *Synechococcus* at 1/6-second intervals, illustrating patterns and speed of movement. Sealed wet mounts of cell suspensions were recorded by phase-contrast video microscopy. (A and B) Strain WH8103. (C and D) Strain WH8113. Filled tracings show the path of a cell where it crosses previous tracings. Average speed between arrows: (B) 13.3 μm/sec and (D) 15.5 μm/sec.

The existence of swimming cyanobacteria will be particularly fascinating if future studies confirm that the swimming strains lack flagella or other external organelles. It is difficult to imagine how a cell the size and shape of *Synechococcus* could be driven through a liquid medium at 25 μm/sec by any other known mechanism.

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Partial Inversion of the Initiation-Promotion Sequence of Multistage Tumorigenesis in the Skin of NMRI Mice

Abstract. *Alterations in NMRI mouse skin induced by the phorbol ester 12-O-tetradecanoylphorbol-13-acetate in "stage I of tumor promotion" are slowly reversible, and this reversibility has a half-time of 10 to 12 weeks. The tumor response observed in the course of an initiation-promotion experiment in vivo is independent of whether stage I of promotion occurs before or after initiation. Since the time interval between treatment with the promoter, and subsequent initiation can be extended up to at least 6 weeks, an enhancement of initiation because of promoter-induced cellular DNA synthesis seems to be unlikely. This result may be inconsistent with the two-stage model of tumor promotion because it indicates that in skin the existence of initiated cells is not required for the induction of cellular alterations that are essential for the stage of skin tumorigenesis called stage I of promotion.*

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The terms initiation and promotion were introduced to distinguish between sequential stages of tumor induction observed under strictly controlled experimental conditions (*1*). Initiation is brought about by treatment of a tissue with a carcinogenic agent at a dose that is insufficient to cause tumor development by itself. Tumors develop in such initiated tissues after long-term application of a promoting agent which, under the conditions of the experiment, does not exhibit tumorigenic potency in non-initiated tissues (*1*).

The temporal sequence of initiation and promotion has been thought to be of critical importance—that is, the experiment could not be done in reverse (*1, 2*). Boutwell (*3*) proposed that in mouse skin the process of promotion itself consists of two different stages. Recently, this concept has gained considerable support by the introduction of "incomplete" tumor promoters. When these substances were used in multistage tumorigenesis experiments in mouse skin, stage I of promotion was brought about by a single treatment with a so-called complete promoter such as the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA), whereas stage II was brought about by long-term treatment of the animals with an incomplete promoter such as 12-retinoylphorbol-13-acetate (RPA) (*4*) or the diterpene ester mezerein (*5*). Whereas promotion had generally been thought to be a fully reversible process, stage I of promotion in the skin of NMRI mice was characterized by persistent effects (*6*). We now report experiments showing that stage I of promotion is slowly reversible and can occur before initiation.

The proof of persistent effects in stage I of promotion was a major prerequisite for the experiments to be described. Therefore, initiation was carried out on the skin of female albino mice (strain NMRI) by a single topical application of 7,12-dimethylbenz[*a*]anthracene (DMBA) in a subthreshold dose. Stage I of promotion was carried out by two topical applications of TPA (or RPA as control) 1 week after initiation. This treatment was followed after different time intervals by repeated applications of the incomplete skin tumor promoter RPA twice weekly for at least 18 weeks (stage II of promotion). As shown in Fig. 1, the effect induced by TPA (stage I) disappeared gradually with a half-time of 10 to 12 weeks. If in stage I, TPA was replaced by RPA, only a slight increase of tumor incidence was observed when the time interval between stage I and stage II was increased. This result indicates that in NMRI mouse skin, RPA-induced stage I promotion became more prominent with time but was still not comparable with the strong effect of TPA within the time interval of our experiment. The persistence of the TPA effect in stage I suggested the possibility of applying TPA a number of weeks before initiation. To permit a complete regression of the short-term TPA effects such as hyperplasia and inflammation, we allowed 2 to 6 weeks to elapse before DMBA was applied. One week after initiation with DMBA, RPA treatment was started.

The tumor rate and tumor yield achieved by this partially inverted approach were almost the same as those obtained with the usual initiation-promotion sequence, whether initiation was carried out by the topical or the intragastral route (Fig. 2). When the animals were treated with RPA instead of with TPA 6 weeks before DMBA application,

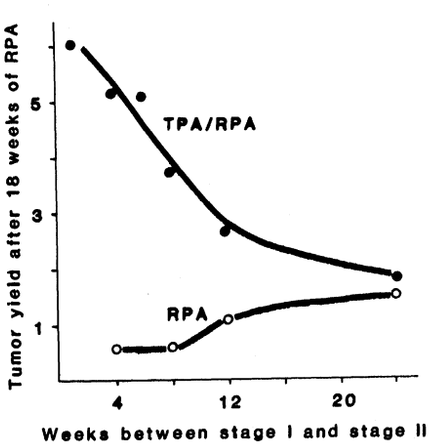

Fig. 1. Two-stage tumor promotion in NMRI-mouse skin—the effect on tumor incidence of increasing the time between stage I and stage II. Female NMRI mice (7 to 8 weeks old; 20 per group) were treated topically with 100 nmol of DMBA dissolved in 100 μl of acetone. Stage I of promotion was carried out with two topical applications of TPA (●) or RPA (○) (20 nmol per 100 μl of acetone) on day 8 and 11 after initiation. For stage II of promotion, long-term RPA treatment (10 nmol per 100 μl of acetone, twice a week) was started at 3, 6, 12, and 24 weeks after stage I treatment (abscissa) and continued for 18 additional weeks. At the end of the experiment, 90 percent or more of the animals were alive. All experiments were repeated twice with similar results. The tumor response is expressed as tumor yield (number of papillomas divided by the number of survivors) after 18 weeks of RPA treatment in stage II.