Photoresponsive Flagellum-Independent Motility of the Purple Phototrophic Bacterium *Rhodobacter capsulatus*

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We report the discovery of photoresponsive, flagellum-independent motility of the α-proteobacterium *Rhodobacter capsulatus*, a nonsulfur purple phototrophic bacterium. This motility takes place in the 1.5% agar-glass interface of petri plates but not in soft agar, and cells move toward a light source. The appearances of motility assay plates inoculated with wild-type or flagellum-deficient mutants indicate differential contributions from flagellar and flagellum-independent mechanisms. Electron microscopy confirmed the absence of flagella in flagellar mutants and revealed the presence of pilus-like structures at one pole of wild-type and mutant cells. We suggest that *R. capsulatus* utilizes a flagellum-independent, photoresponsive mechanism that resembles twitching motility to move in a line away from the point of inoculation toward a light source.

The movements of microbes in response to illumination have been studied for more than 100 years (6, 8). Purple phototrophic bacteria in liquid media reverse the direction of flagellum-driven movement in response to a sudden change in light intensity. This “Schreckbewegung” or “scotophobic” response occurs regardless of the direction of the light source, which is not phototaxis (2, 8, 16). Experiments on *Rhodobacter sphaeroides* indicated that this response to a change in light intensity requires the light-dependent catalytic activity of the photosynthetic apparatus, but the signal transduction pathway that connects this primary sensation to flagellum rotation is not known (3). The *ctrA* gene of *Rhodobacter capsulatus* is indirectly associated with this response, because the CtrA protein (a response regulator homologue) is a class I flagellar protein required for class II, III, and IV flagellar gene transcription and, hence, for flagellum-dependent motility in liquid or soft agar media (10). In contrast to individual cell movement in liquid media, the purple phototrophic bacterium *Rhodospirillum centenum* exhibits phototaxis of colonies on soft (0.8%) agar, driven by a lateral flagellum (14). Such photoresponsive behavior in purple phototrophic bacteria was thought to be unique to *R. centenum* (8, 16), although some species of cyanobacteria are capable of phototaxis (5, 7).

The motility terms “twitching” and “gliding” are used to describe flagellum-independent bacterial cell movements that take place on solid substrates, sometimes detected in the interface between an agar medium and the underlying surface of a petri plate (12). The twitching motility of *Myxococcus xanthus* and other bacteria appears to be driven by the extension and retraction of type IV pili, whereas the adventurous gliding motility of *M. xanthus* is thought to depend on slime secretion (12, 15). Until now, no member of the α-proteobacteria was reported to be capable of flagellum-independent motility (12, 15).

This paper describes experiments on wild-type and flagellum-deficient mutant strains of the nonsulfur purple α-proteobacterium *R. capsulatus*, which revealed long-range, light-directed movements of cells in the interfacial region between the bottom of an agar medium and the enclosing petri plate. The movements of wild-type and flagellum-deficient strains were qualitatively different, which is attributed to differential contributions from flagellar and nonflagellar mechanisms. Although further experimentation is needed to elucidate the mechanistic details of flagellum-independent motility in *R. capsulatus*, the data presented below are consistent with the existence of a twitching type of motility, as in chemotrophic bacteria and cyanobacteria (5, 7, 12, 15).

**Photoresponsive movement of wild-type strain B10.** To determine whether *R. capsulatus* is capable of motility in the interface of a petri plate, we employed an assay in which a sterile toothpick was dipped into cell pellets obtained by centrifugation of liquid cultures and used to inoculate 1.5% agar-solidified glass plates of YPS medium (0.3% yeast extract, 0.3% peptone, 1 mM CaCl₂, and 1 mM MgSO₄) (20). Plates were inoculated by stabbing vertically through the agar to make contact with the center of the enclosing plate. Stabbed plates were incubated in transparent polycarbonate anaerobic jars (BBL), which were immersed in temperature-controlled (32℃) circulating water in a glass aquarium, and illuminated (intensity of ~190 µE·m⁻²·s⁻¹) from one side by halogen lamps (Capsylite, Sylvania) for photosynthetic growth (19).

Our expectation was that cells would grow on the surface of the agar to form a colony at the point of inoculation and within the agar along the stab line. It was anticipated that if *R. capsulatus* were capable of motility in this assay, a relatively large-diameter, circular zone of growth would be present in the interface between the bottom of the agar-solidified medium and the circular, glass plate, as in the appearance of plates stabbed with chemotrophic bacteria capable of twitching motility (12, 18). We were surprised to observe the movement of the wild-type strain B10 (11) in the agar-plate interface of stabbed plates, which extended from the stab to the edge of the...
frame annotations were obtained from the ERGO Light database. The cheL site (108th codon), and the wild-type gene of strain B10 was replaced with this neo gene of the KIXX cassette (4) into the fliC HindIII site (108th codon), and the wild-type gene of strain B10 was replaced with this fliC disruption using GTA transduction (17). Gene and open reading frame annotations were obtained from the ERGO Light database. The cheL sequence is homologous to cheL of Caulobacter crescentus (9).

plate nearest the light source (Fig. 1a). There was a line of cells surrounded by a broad zone of cell movement, as well as discrete colonies, all located within the interface between the agar medium and the enclosing plate.

Photoresponsive movement of flagellar mutant strains. Previous experiments showed that flagellum-dependent motility of R. capsulatus in liquid or soft agar (0.4%) media is impaired in the ctrA mutant strain BCKF (10). When BCKF was evaluated in the agar plate stab assay, a relatively narrow line of cell movement toward the light was seen (Fig. 1b). Although these data show that CtrA-independent motility drives photoresponsive movement of cells, it was conceivable that this movement was driven by flagellar motility. This is because the environmental signal that results in CtrA activation is not known, and the agar-plate interface may present an environment that allows flagellar gene expression in the absence of CtrA, in contrast to previously studied cultures in liquid and soft agar media (10).

The nearly complete genome sequence (nine contigs) of R. capsulatus (ERGO Light database) encodes a single flagellin homologue (orf 03417, annotated as fliC), which is located in a cluster of putative flagellar genes (Fig. 2). Because we wished to study a mutant that is incapable of flagellum formation under all growth conditions, the fliC gene of the wild-type strain B10 was disrupted (Fig. 2). The resultant fliC mutant was designated KSHF1 and, like the ctrA mutant, was deficient in motility in soft agar stabs (Fig. 3). The appearance of this fliC mutant in the illuminated agar plate stab assay of motility was the same as the ctrA mutant (compare Fig. 1b and c). Thus, this light-directed motility is independent of the flagellin encoded by the fliC gene.

Electron microscopy of wild-type and flagellar mutant strains. Although the available genome sequence does not contain additional flagellar gene homologues, it was conceivable that a second set of flagellar genes is absent from the nine contigs that have been sequenced. Therefore, we examined the wild-type, ctrA, and fliC strains by transmission electron microscopy to evaluate the presence of flagella. Cells were taken from the agar-plate interface after movement and negatively stained with 2% uranyl acetate for 1 min. As shown in Fig. 4, the wild-type strain contained a flagellum, which was absent from the flagellar mutants. However, the wild-type strain and the two flagellar mutants had what appeared to be pili extending from a single pole of these rod-shaped cells.

The R. capsulatus genome sequence lacks a pilA (pilin gene) homologue, although BLAST (1) analysis indicated several weak homologues of sequences (PilB, orf 00409, annotated as a hypothetical membrane-spanning protein; PilQ, orf 03968, annotated as a general secretion pathway protein D; and PilU, orf 03965, annotated as a secretory protein kinase) that have been implicated in the twitching motility of other bacteria (12, 13, 15). We found that alignments of these R. capsulatus sequences to genuine type IV pilus-related proteins yielded identities of only 15 to 24% (data not shown). This is consistent with the suggestion that α-proteobacteria lack the conserved core of type IV pilus genes (15), and so the mechanism of flagellum-independent motility of R. capsulatus may differ from type IV pilus-driven motility.

Velocity of mass cell movement and the possibility of convection. It was found that cells move from the central point of inoculation to the wall of 3.5-cm-radius plates within 20 h,
which indicates a minimum motility velocity of approximately 29 \( \mu \text{m} \cdot \text{min}^{-1} \). Thus, the velocity of cell movement is greater than could be obtained by directional cell division, because in this medium \( \textit{R. capsulatus} \) cells divide every 2 to 3 h; assuming a maximal cell length of 10 \( \mu \text{m} \), directional cell division would result in an apparent movement of \(<0.1 \mu \text{m} \cdot \text{min}^{-1} \). Although the velocities of gliding and twitching motility in other bacteria vary greatly (e.g., 1.5 to 600 \( \mu \text{m} \cdot \text{min}^{-1} \), depending on the species and the mechanism) (12, 13, 15), the velocity of \( \textit{R. capsulatus} \) flagellum-independent movement is within this range.

The possibility of cell convection was tested by use of formaldehyde-killed cells (labeled with 50 \( \mu \text{Ci} \cdot \text{ml}^{-1} \) of \([32P]\)orthophosphate during growth in liquid YPS medium and detected by autoradiography of stabbed plates). As shown in Fig. 5, the movement of live \( \textit{ctrA} \) mutant cells was detected, whereas the formaldehyde-killed cells did not appear to move beyond the point of inoculation. The possible influence on the direction of motility by a magnetic field, gravity, temperature gradients, and reuse of plates all yielded negative results. Therefore, the direction of flagellum-independent cell movement shown in Fig. 1 and 5 appears to be due solely to a light signal specified by the direction of illumination.

Concluding remarks. Our results show that \( \textit{R. capsulatus} \) is capable of flagellum-independent locomotion, which was previously thought to be absent from the \( \alpha \)-proteobacteria (12, 15). We suggest that \( \textit{R. capsulatus} \) motility within the agar-plate interface is driven by both flagellar and flagellum-independent locomotion, which are superimposed in the wild-type strain. The flagellar component of motility is independent of the direction of illumination and broadens the flagellum-independent movement, whereas the flagellum-independent locomotion results in movement toward a light source. Perhaps the pilus-like structures seen in electron micrographs drive flagellum-independent motility.

It is unclear why some apparently flagellum-driven cells of the wild-type strain break away from the cell mass, only to stop and form a colony (Fig. 1a). Flagellum-driven cells may occasionally escape from a critical mass of cells that is required for flagellum-independent movement toward the light source. It was noted that a critical cell density is usually required for mass cell twitching motility in other bacteria (12). However, it would be thought that this critical cell density would be reached during cell division to form a colony. Instead, these colonies may indicate heterogeneity in the cell population or a response to spatial variation in a physical or chemical aspect of the agar-plate interface.

The stabbed agar plate assay was also used to discover photosensitive mass cell movement of the purple phototrophic bacteria \( \textit{Rubrivivax gelatinosus} \), \( \textit{Rhodopseudomonas palustris} \), and \( \textit{Blastochloris viridis} \) (data not shown), and so such light-responsive behavior is not limited to \( \textit{R. capsulatus} \) and \( \textit{R. centenum} \). It will be interesting to follow up on the experiments reported in this paper to determine the mechanism of motility in the \( \textit{R. capsulatus} \) flagellar mutants and the pathway of light signal transduction that specifies the direction of movement.

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FIG. 5. Agar plates stabbed with \(^{32}P\)-labeled \( \textit{ctrA} \) mutant (BCKF) cells and incubated anaerobically with illumination for 2 days. (a) Autoradiograph of a plate stabbed with live cells. (b) Autoradiograph of a plate stabbed with formaldehyde-killed cells (a liquid culture was treated with 7% formaldehyde for 1.5 h on ice before pelleting cells and stabbing the plate). (c) Photograph of the plate shown in panel a. (d) Photograph of the plate shown in panel b. The live-cell control shown in panels a and c was treated identically to the formaldehyde-killed cells except that water was added in place of formaldehyde. The direction of illumination was from the top of the figure.