Short Communication

Characterization of myxobacterial A-motility: insights from microcinematographic observations

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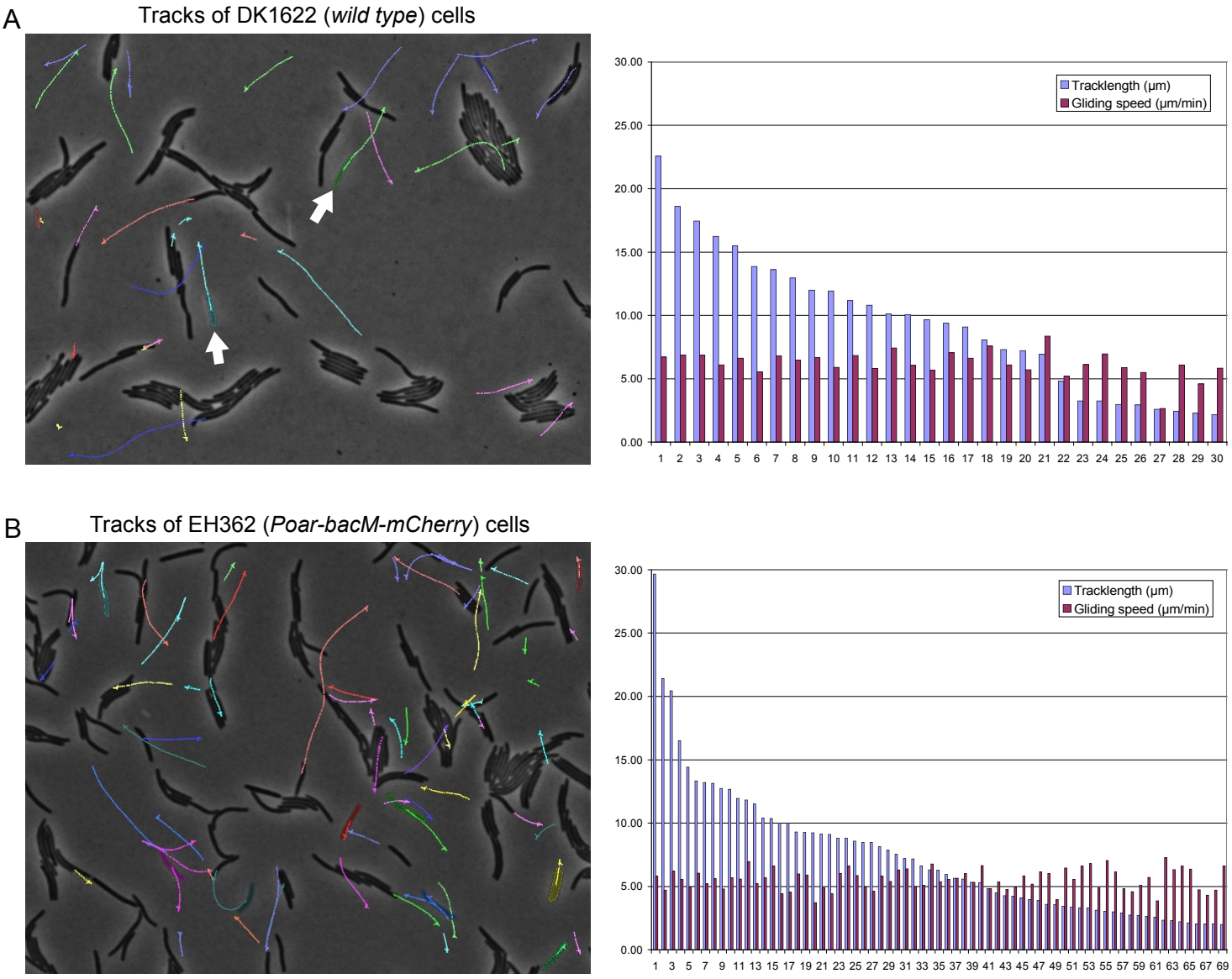

Figure S1. Tracking of *M. xanthus* cells gliding over TPM agar patches. (A) DK1622 cells. Shown is the first of 300 phase contrast images onto which all recorded tracks obtained from the complete 10 min video sequence are overlaid. In the adjacent graph, the recorded gliding speeds are displayed arranged by decreasing track length. Large white arrows point to two of the tracked cells and the corresponding tracks registered for their centroids. (B) EH362 cells, displayed and analyzed as in A. Note that there is no correlation between gliding speeds and the length of the recorded tracks.

Gliding speed analysis:

Cells were grown on CTT agar plates for 9 days, resuspended to an OD of 0.1 in TPM buffer, and 1 µl cell suspension was spotted onto a TPM agar patch and covered with a cover slip. The edges of the slip were sealed with paraffin/vaseline (2:1) and the cells were incubated for 48 hours at 25ºC. Videos were recorded at 30 exposures per minute for 10 minutes using a Nikon Eclipse 90i microscope with a 100x oil immersion phase contrast objective controlled by Volocity software (Improvision).

Tracking data were generated from these videos using Volocity software. Objects were selected by intensity to select all cells while excluding the surrounding agar surface, and by size to include only objects > 2 µm² and < 7 µm², resulting in the selection of only single isolated cells. Furthermore, objects touching the edges of the image were excluded. Tracking of the selected objects was performed with the settings "Shortest distance" and using 1 µm as "maximum distance between objects". After eliminating all tracks of less than 2 µm length, the data were exported into an Excel file and analyzed. To improve the reliability of the tracking data, cells with speeds of less than 1 µm/min, or with excessively high speeds (resulting from tracking errors) were excluded from the analysis; these corrections did not change the calculated average speeds by more than 0.1 to 0.2 µm/min.

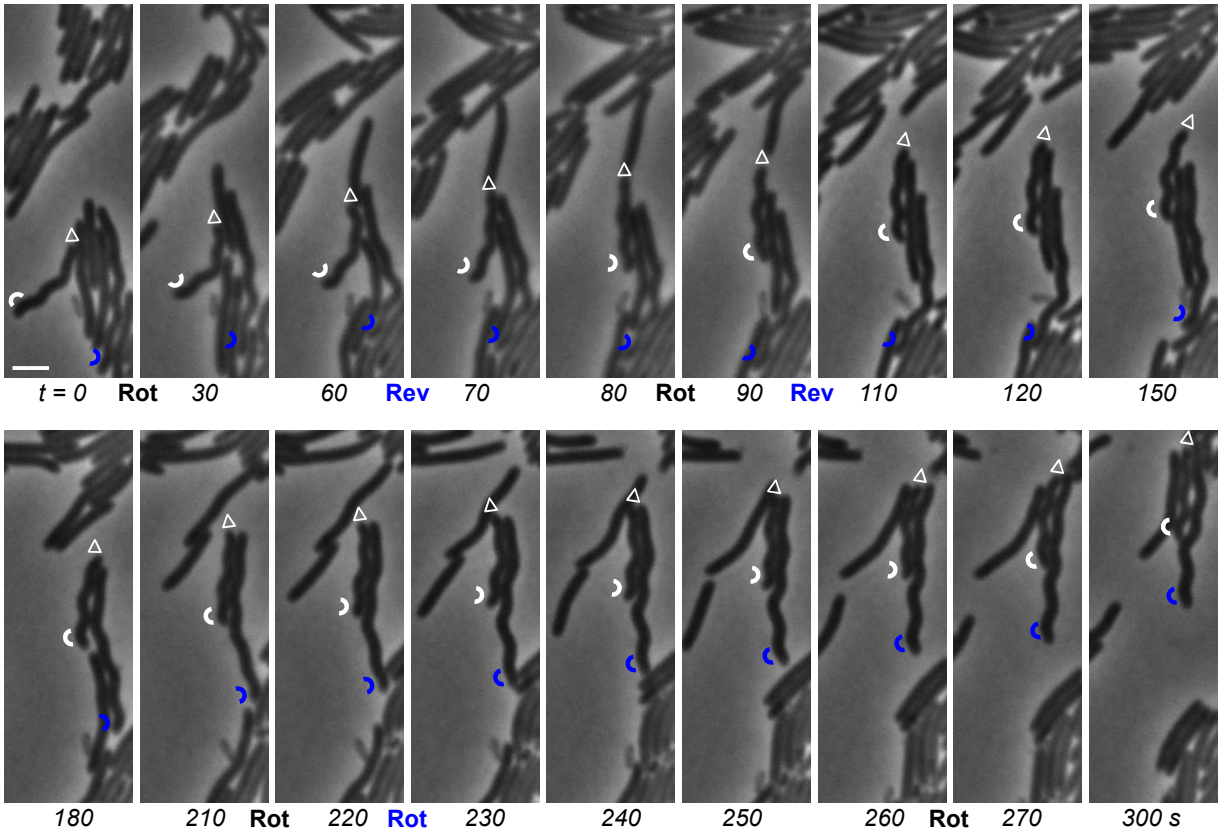

Figure S2. EH302 cells (Δ*pilQ*, Δ*bacM*) gliding over a TPM agar patch 48 hours after spotting. For EH302 cells gliding in groups, rotations are occasionally seen during cell-cell contacts. Arrowheads point out the direction of gliding for a cell that undergoes several rotations (Rot) while contacting neighboring cells. Cell orientations are indicated for two cells by white and blue arcs, respectively, present near one of each cell's poles. A change of arc direction by 180 degrees indicates a cellular rotation of 180 degrees. In the case of the cell marked by blue arcs, only one rotation is seen per 10.4 µm gliding (between 110 and 300 s). Reversals of gliding direction are indicated by "Rev". This result demonstrates that rotations can be detected in this experimental setup in case they are occurring.

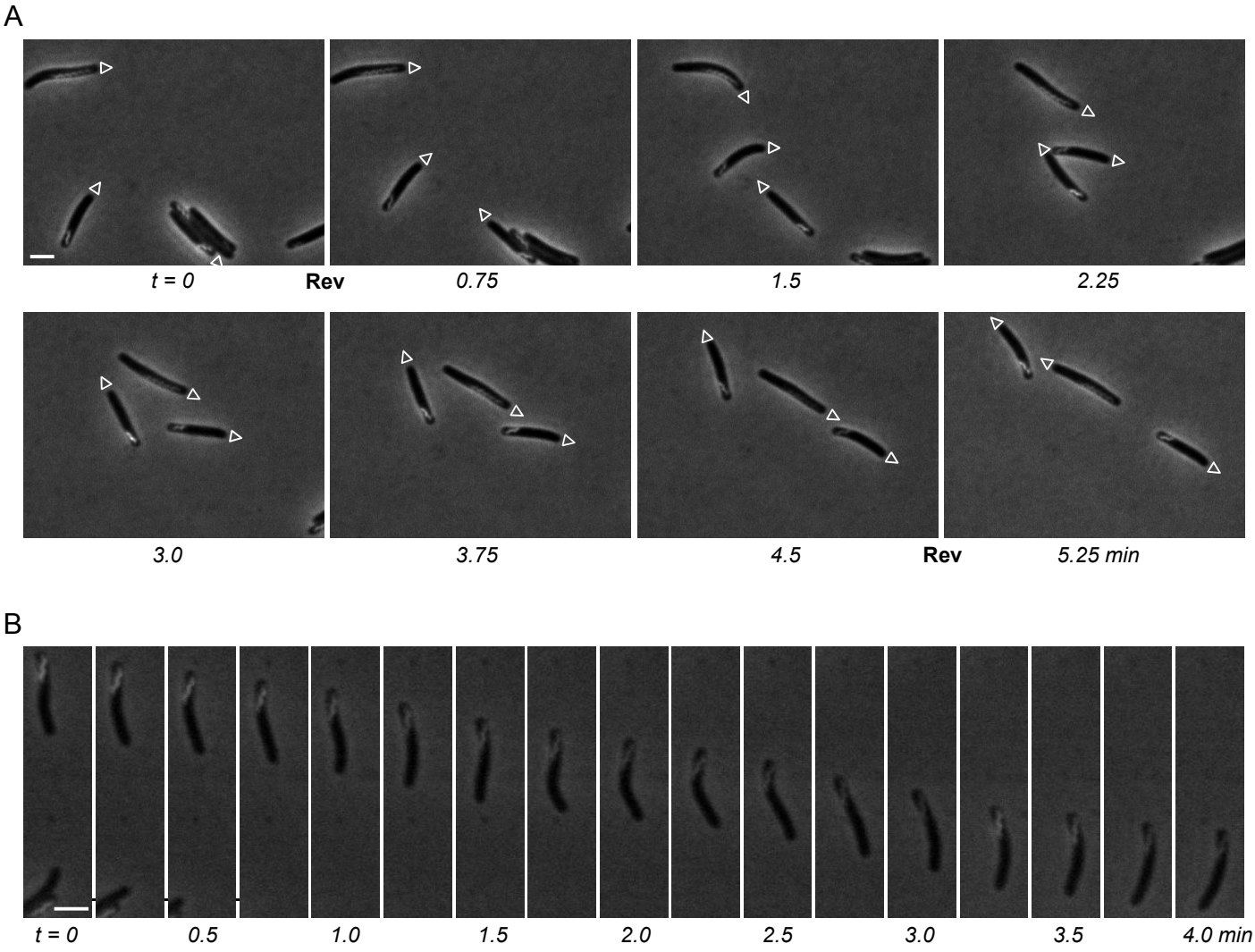

Figure S3. Gliding of cells producing a BacM-mCherry fusion protein, which forms a fluorescent screw near one of the cell poles. (A) Three cells of strain EH362, which overexpresses *bacM-mCherry* while *bacM* is present in the wild-type operon; 48 hours after spotting on TPM agar. Reversals of gliding direction are indicated by "Rev"; arrowheads mark the gliding direction of individual cells. (B) Cell of S-motility-deficient strain EH367, which expresses *bacM-mCherry* in the absence of *bacM* and *pilQ;* 60 hours after spotting on TPM agar.
Note that none of the screws changes its relative intracellular orientation during gliding in any of the cells. Gliding is recorded under phase contrast while the mCherry fluorescence is concomitantly recorded. White bars represent 2 µm.

Supplementary Movies:

Movie 1A: EH302 cells (Δ*pilQ*, Δ*bacM*) after 24 hours on TPM agar patch. Original image dimensions 13 x 32.5 µm (200 x 500 px). Video was recorded for 8 min at 60 exposures per minute (480 exposures) and is shown at ~ 30 frames per second for 16 sec (480 frames).

Movie 1B: EH302 cells (Δ*pilQ*, Δ*bacM*) after 24 hours on TPM agar patch. Original image dimensions 13 x 13 µm (200 x 200 px). Video was recorded for 7.5 min at 60 exposures per minute (450 exposures) and is shown at ~ 30 frames per second for 15 sec (450 frames).

Movie 2A: EH362 cells (*Poar-bacM-mCherry*) after 48 hours on TPM agar patch. Original image dimensions 6.5 x 19.5 µm (100 x 300 px). Video was recorded for 4.5 min at 20 exposures per minute (90 exposures) and is shown at ~ 30 frames per second for 3 sec (90 frames).

Movie 2B: EH367 cells (Δ*pilQ*, Δ*bacM, Poar-bacM-mCherry*) after 60 hours on TPM agar patch. Original image dimensions 6.5 x 11 µm (100 x 170 px). Video was recorded for 4 min at 60 exposures per minute (240 exposures) and is shown at ~ 30 frames per second for 9 sec (240 frames).

Movie S2: EH302 cells (Δ*pilQ*, Δ*bacM*) after 48 hours on TPM agar patch. Original image dimensions 6.5 x 19.5 µm (100 x 300 px). Video was recorded for 5 min at 60 exposures per minute (300 exposures) and is shown at ~ 30 frames per second for 12 sec (300 frames).

Movie S3A: EH362 cells (*Poar-bacM-mCherry*) after 48 hours on TPM agar patch. Original image dimensions 19.5 x 26 µm (300 x 400 px). Video was recorded for 6 min at 20 exposures per minute (120 exposures) and is shown at ~ 30 frames per second for 4 sec (120 frames).

Movie S3B: EH367 cells (Δ*pilQ*, Δ*bacM, Poar-bacM-mCherry*) after 60 hours on TPM agar patch. Original image dimensions 3.9 x 15.6 µm (60 x 240 px). Video was recorded for 4 min at 60 exposures per minute (240 exposures) and is shown at ~ 30 frames per second for 9 sec (240 frames).

