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## Supplemental Information

# The Second Messenger c-di-GMP Adjusts Motility and Promotes Surface Aggregation of Bacteria

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## Supporting Material

**Viscosity of different concentrations of Ficoll:** The viscosity coefficients of different concentrations of Ficoll solution was measured by a viscosity meter (NDJ-5s; Shanghai Pingxuan Scientific) at room temperature of 23 °C. The viscosities of Ficoll at different concentrations (15, 12, 9, 7, 5, 3, 2 and 0%) are: 9.99 cp, 6.71 cp, 4.24 cp, 3.00 cp, 2.19 cp, 1.60 cp, 1.34 cp, 0.99 cp. The values are similar to that measured by Chen and Berg (ref. 16).

Table S1. Strains and plasmids used in this work.

| Strain | Relevant genotype | Plasmids | Assay |
|---|---|---|---|
| JY27 | Δ*fliC cheY* | pKAF131 (*fliC*$^{st}$) | Torque-speed |
| RW1 | Δ*fliC cheY yhjH* | pKAF131 | Torque-speed |
| RW3 | Δ*fliC cheY yhjH ycgR* | pKAF131 | Torque-speed |
| JY27 | Δ*fliC cheY* | pKAF131<br>pWB5 (*cheY*) | CCW vs CW rotation |
| RW1 | Δ*fliC cheY yhjH* | pKAF131<br>pWB5 | CCW vs CW rotation |
| RW3 | Δ*fliC cheY yhjH ycgR* | pKAF131<br>pWB5 | CCW vs CW rotation |
| JY26 | Δ*fliC* | pKAF131 | Rotational bias |
| RW2 | Δ*fliC yhjH* | pKAF131 | Rotational bias |
| RW4 | Δ*fliC yhjH ycgR* | pKAF131 | Rotational bias |
| JY26 | Δ*fliC* | pBAD33*fliC* | Near surface |
| RW2 | Δ*fliC yhjH* | pBAD33*fliC* | Near surface |
| RW1 | Δ*fliC cheY yhjH* | pKAF131<br>pHS1 (Proteorhodopsin) | Resurrection experiment |
| RW3 | Δ*fliC cheY yhjH ycgR* | *efgp-ycgR* on pTrc99a | Dynamic fluorescent experiment |
| RW2 | Δ*fliC yhjH* | pJY7 (*motAmotB*)<br>pFD313 (*fliC*$^{st}$) | Overproduction of MotA MotB |

## Supporting figures

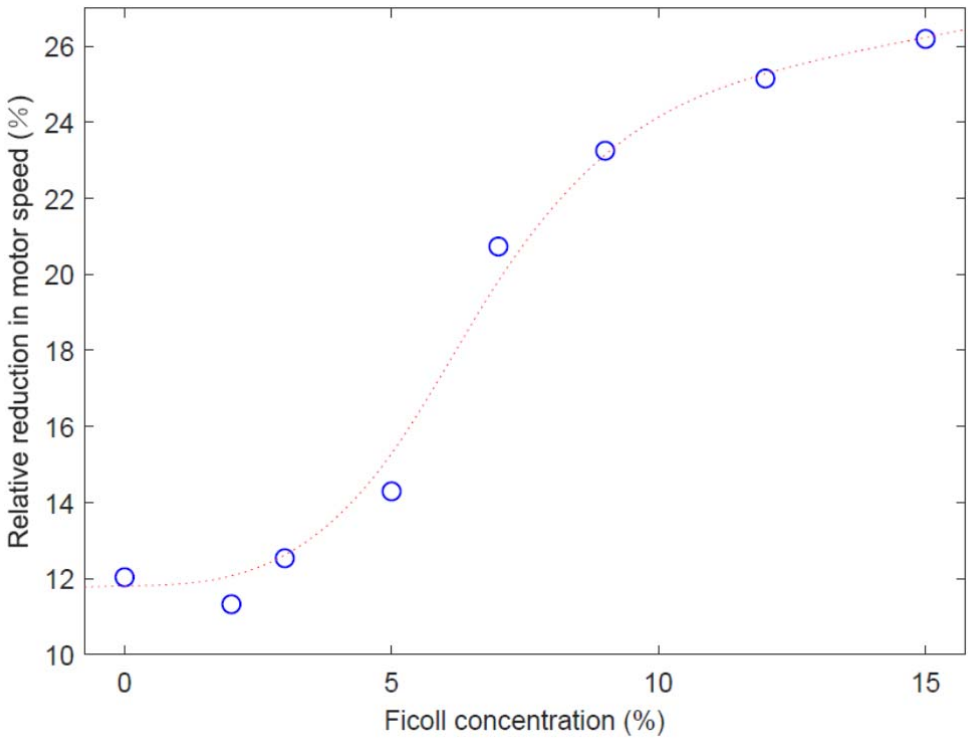


Fig.S1. Relative reduction in motor speed with elevated c-di-GMP level for motors labeled with 0.35-μm-diameter latex beads in different concentrations of Ficoll solution. The red dashed line is a spline fitting to guide the eye.

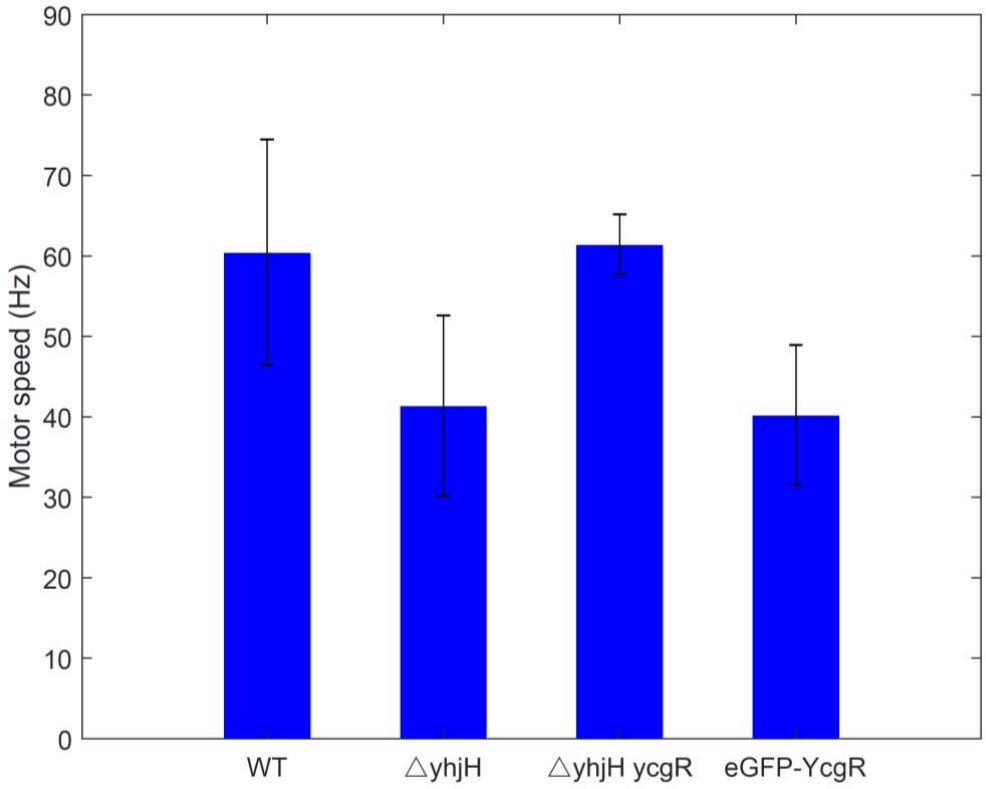


Fig.S2. Motor speed of different strains with 1.0 μm diameter latex bead. From left to right: JY27, RW1, RW3, and RW3 transformed with pTrc99a-eGFP-YcgR. The bars and errors are means and standard deviations. (The number of motors measured were 43, 37, 39 and 41, respectively.)

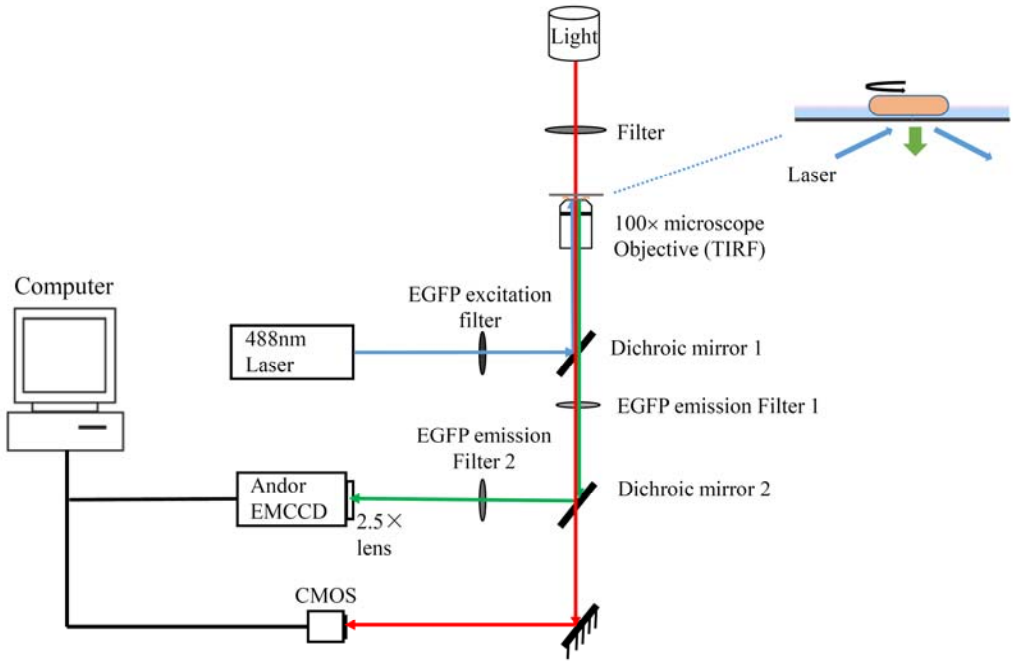


Fig.S3. Schematic diagram of the TIRF setup used for viewing eGFP-YcgR localization with TIRF and tether-cell rotation with phase-contrast simultaneously.

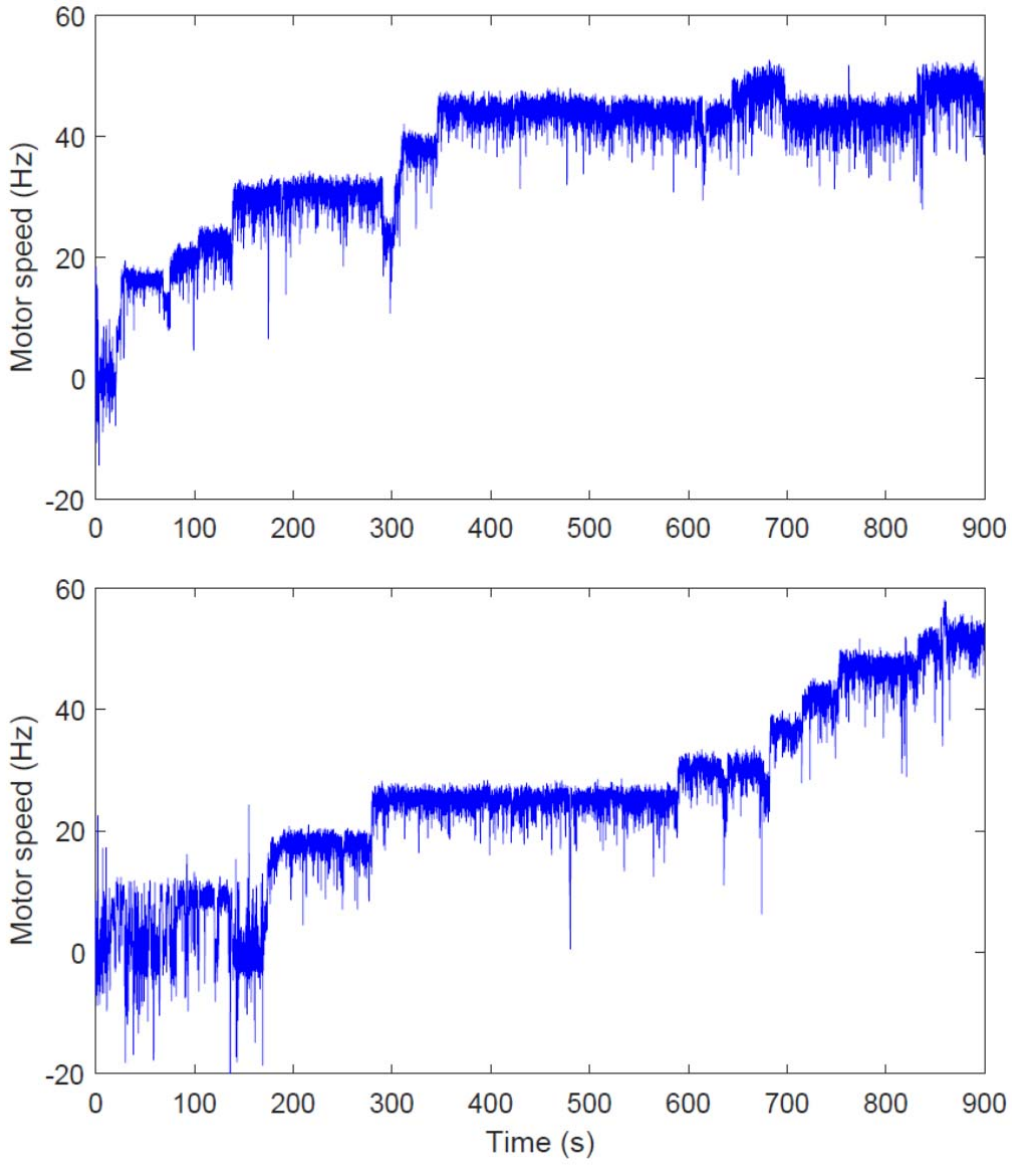


Fig. S4. Sample traces of motor resurrection at high load (1-μm-diameter latex bead). 20 mM $NaN_3$ was flowed for 30 min to deplete PMF, and PMF was then restored at time zero.