

Measurements of Free-swimming Speed of Motile *Salmonella* Cells in Liquid Media

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[Abstract] Bacteria such as *Escherichia coli* and *Salmonella enterica* swim in liquid media using the bacterial flagella. The flagellum consists of the basal body (rotary motor), the hook (universal joint) and the filament (helical screw). Since mutants with a defect in flagellar assembly and function cannot swim smoothly, motility assay is an easy way to characterize flagellar mutants. Here, we describe how to measure free-swimming speeds of *Salmonella* motile cells in liquid media. Free-swimming behavior under a microscope shows a significant variation among bacterial cells.

Keywords: Bacterial flagella, Motility, Motor, Optical microscopy, Proton motive force, *Salmonella*

[Background] The flagellar motor of *E. coli* and *Salmonella* is powered by downhill proton translocation along proton motive force (PMF) across the cytoplasmic membrane (Morimoto and Minamino, 2014; Minamino and Imada, 2015). The rotational speed of the proton-driven flagellar motor is proportional to total PMF (Gabel and Berg, 2003). Therefore, measurements of free-swimming speeds of motile cells allow us not only to analyze motor performance of various mutants but also to examine whether there is a significant difference in total PMF under experimental conditions (Minamino *et al.*, 2016).

Materials and Reagents

1. 1.5 ml Eppendorf tubes
2. Double-sided tape (NICHIBAN, catalog number: NW-5)
3. Glass slide (Matsunami Glass, catalog number: S1126)
4. 18 x 18 mm coverslip (thickness: 0.12-0.17 mm) (Matsunami Glass, catalog number: C018181)
5. Pipette tips
6. Filter paper
7. *Salmonella* SJW1103 strain (wild-type for motility and chemotaxis) (Yamaguchi *et al.*, 1984)
8. *Salmonella* MMHI0117 strain [Δ *fliH-fliI fliB*(P28T)] (Minamino and Namba, 2008)
9. Bacto tryptone (BD, catalog number: 211705)
10. Potassium dihydrogenphosphate (Wako Pure Chemical Industries, catalog number: 164-22635)
11. Dipotassium hydrogenphosphate (Wako Pure Chemical Industries, catalog number: 164-04295)
12. Bacto yeast extract (BD, catalog number: 212750)
13. Bacto agar (BD, catalog number: 214010)
14. Sodium chloride (Wako Pure Chemical Industries, catalog number: 192-13925)

15. T-broth (TB) (see Recipes)
16. L-broth agar plate (see Recipes)

Equipment

1. Selection of single channel pipettes (1,000 μ l, 100 μ l) (Gilson, model: P-1000, P-100)
2. Shaking incubator (30 °C, at 200 rpm) (TAITEC, model: BR-40LF)
3. Centrifuge (able to hold 1.5 ml tube, spin at 6,000 \times g) (TOMY SEIKO, model: MX-305)
4. Spectrophotometer (able to measure OD₆₀₀) (GE Healthcare, model: GeneQuant 1300)
Note: This product has been discontinued by the manufacturer.
5. Phase contrast microscope (Olympus, model: CH40)
 - a. 40x objective lens
 - b. CCD camera (Hamamatsu Photonics, model: C5405)
6. Objective micrometer (10 μ m/pitch)
7. Hard-disk video recorder (Panasonic, model: DMR-XP25V)

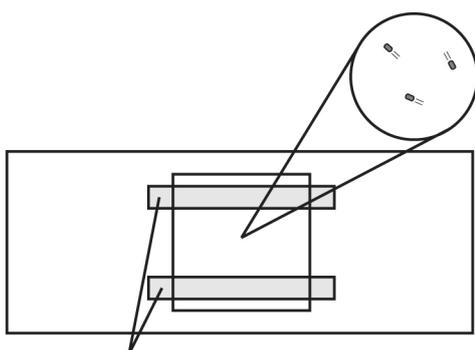
Software

1. Move-tr/2D (Library Co., Tokyo)
2. Microsoft Excel (Microsoft)

Procedure

Note: Carry out procedures at ca. 23 °C unless otherwise specified.

1. Pick a single colony from L-broth agar plate and inoculate it into 5 ml of fresh TB.
2. Incubate overnight at 30 °C with shaking at 150 rpm.
3. Inoculate 50 μ l of overnight culture of *Salmonella* SJW1103 and MMHI0117 strains into 5 ml of fresh TB and incubate at 30 °C for 5 h with shaking at 150 rpm. (The cell density reaches an OD₆₀₀ of ca. 1.0-1.2.)
4. Transfer 100 μ l of the culture into a 1.5 ml Eppendorf tube.
5. Collect the cells by centrifugation (6,000 \times g, 2 min).
6. Suspend the cell pellet in 1.0 ml of fresh TB.
7. Centrifuge at 6,000 \times g for 2 min.
8. Discard supernatant.
9. Repeat steps 4-6.
10. Resuspend the cells in 1.0 ml of fresh TB.
11. Make a tunnel slide by sandwiching double-sided tape between glass slide (bottom side) and 18 x 18 mm coverslip (top side) (see Figure 1 and Video 1).



double-sided tape

Figure 1. Tunnel slide for observation of bacteria cells under optical microscopy. Double-sided tapes are sandwiched between a glass slide (bottom side) and an 18 x 18 mm coverslip (top side). Cells are added to the space between the glass slide and the coverslip. Observation area is magnified in a circle.

Video 1. Preparation for a tunnel slide



12. Add the cell suspension to the tunnel slide and absorb excess medium with a piece of filter paper.
13. Set the tunnel slide on the stage of a phase contrast microscope.
14. Select a 40x objective lens.
15. Observe motile cells under the phase contrast microscope.

Video 2. Free-swimming of *Salmonella* cells



16. Record a movie of motile cells for 10-30 sec with a hard-disk video recorder.
17. Move a field of view under the microscope.
18. Repeat steps 14 and 15 more than 5 times.
19. Capture an image of an objective micrometer in the same setting.
20. Transfer the movies recorded on the hard-disk video recorder to a DVD.

Data analysis

1. Calibrate a scale of a movie using the image of the objective micrometer.
2. Track motile cells in the movie with the length of 1 sec (30 sequential images recorded at 30 frames per second) using a motion analysis software Move-tr/2D (Library Co., Tokyo).
3. Measure the velocity ($\mu\text{m}/\text{sec}$) of each cell by the Move-tr/2D.
4. Calculate the average velocity and standard deviation from the data of more than 30 cells using Microsoft Excel (Microsoft).

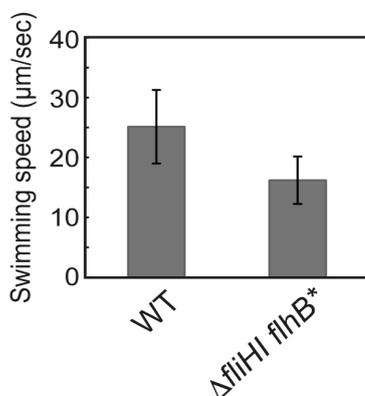


Figure 2. Free-swimming speed of *Salmonella* cells. SJW1103 (WT) and MMHI0117 ($\Delta\text{fliH1 flhB}^*$) were grown at 30 °C for 4 h in T-broth and observed by a phase contrast microscope. Vertical bars indicate standard deviations. (Modified from Minamino *et al.*, 2016)

Notes

1. When free swimming of motile cells is observed by phase contrast microscopy, a proper cell concentration is required. If the cell concentration is low, only a small number of cells are observed in a single movie. If the cell concentration is quite high, traces of each motile cell are overlapped, thereby making precise cell tracking difficult. The free-swimming motility is not affected at all by cell-cell interactions.
2. Cells nonspecifically attached on a glass surface are often observed and so cannot show any free-swimming motility. So these cells must not be judged as non-motile cells.

Recipes

1. T-broth (TB)
1% Bacto tryptone, 10 mM potassium phosphate, pH 7.5
2. L-broth agar plate
1% Bacto tryptone, 0.5% Bacto yeast extract, 1% NaCl, 1.5% Bacto agar

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