Distinct chemotactic behavior in the original *Escherichia coli* K-12 depending on forward- and-backward swimming, not on run-tumble movements

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Supplementary Figure 1 Characterization of swimming motility and structural parameters of SHU101

 (a) *Left*: Phase-contrast image of the non-flagellated mutant, SHU101. Scale bar, 50 μm. *Right*: The sequential phase-contrast images with 165-ms intervals were integrated for 5 29 s with the intermittent color code 'red, \rightarrow yellow, \rightarrow green, \rightarrow cyan, \rightarrow blue.' The processive linear movements could not be seen, indicating that the SHU101 is a non- motile strain. (b) Motility of SHU101 cells on a 0.25 % (wt/vol) soft-agar plate at 30ºC for 7 h. Scale bar, 0.5 cm. (c) Electron micrograph. Scale bar, 2 μm.

36 **Supplementary Figure 2 Quantification of rotational rate and structural** 37 **parameters of flagella under total internal reflection fluorescence** 38 **microscope (TIRFM)**

39 (a) Histograms of the rotation rate and structural parameters during CCW (*top*) and CW 40 rotation (*bottom*) in ATCC10798 cells. Solid line represents the Gaussian fitting. The 41 peaks and SDs of rotational rates were 73.3 ± 32.8 Hz in CCW direction (n = 74) and 42 72.4 \pm 23.7 Hz in CW direction (n = 39). The flagellar pitches were 1.1 \pm 0.1 µm in CCW 43 (n =142) and 1.1 \pm 0.1 µm in CW direction (n = 80). The pitch angles were 38.6 \pm 5.4 44 degree in CCW (n = 70) and 40.5 ± 4.5 degree in CW (n = 39). The helix radii were 0.14 45 ± 0.02 μm in CCW (n = 142) and 0.14 ± 0.01 μm in CW (n = 80). (b) Histograms of the 46 structure and rotation rate of flagella of W3110 during left-handed (*top*) and right-handed 47 *(bottom)* state. The rotation rates were 87.6 ± 34.0 Hz under a left-handed state (n = 133) 48 and 81.5 \pm 22.7 Hz under a right-handed state (n = 42). The flagellar pitches were 2.3 \pm 49 0.2 μm under a left-handed state (n = 112) and 1.1 ± 0.1 μm under a right-handed state (n 50 = 84). The pitch angles were 30.5 ± 4.2 degree under a left-handed state (n = 81); 36.2 \pm 51 4.8 and 53.2 ± 5.1 degree under a right-handed state (n = 40). The helix radii were 0.21 \pm 52 0.03 μm under a left-handed state (n = 112); 0.13 ± 0.02 μm and 0.23 ± 0.04 μm under a 53 right-handed state $(n = 84)$.

Supplementary Figure 3 Real-time imaging of flagellar polymorphism in W3110

 Sequential images of the flagellar polymorphism at 2.5-ms intervals. *Left*: The orientation of flagellar filament(s) was from the first quadrant to the third quadrant relative to the major axis of the filament, indicating that the helicity of filaments was left-handed. From 0 to 22.5 ms, the wave of flagella propagated in a direction away from the hook end toward the flagellar tip, indicating that the flagella rotated in CCW direction. The direction of rotation was switched at 30.0 ms, and then their helicity changed from the left-handed into right-handed within 50 ms. Taken together that the pitch angle of flagellar filaments was 58 degrees, the filament form was the semi-coiled. Scale bar, 2 μm. *Right*: The orientation of flagellar filament (s) was from the second quadrant to the fourth quadrant relative to the major axis of the filament, indicating that the helicity of filaments was right-handed. We concluded that the flagellar form was the curly state with 68 the fact of the 40° -pitch angle. From 0 to 17.5 ms, the flagellar filament did not move. At 20 ms, the flagellar filaments gradually moved, suggesting that the flagella started to rotate. The flagellar filaments dynamically twisted at 40 ms, and then the right-handed and left-handed flagellar were combined into a single filament at 67.5 and 105.0 ms, which was also seen in another bacterium. Finally, the curry filament(s) transformed into the normal state within 100 ms. Scale bar, 2 μm. Data from Supplementary Videos 6 and 7.

Supplementary Figure 4 Quantification of flagellar radius at coiled state in W3110

(a) The fluorescent micrograph of coiled-state flagellar filaments. Scale bar; 3 μm. (b)

Histogram of the radius of coiled flagella. The solid line represents the Gaussian fitting,

- 80 where the peak and SD are 0.78 ± 0.02 µm (n = 39).
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Supplementary Figure 5 Chemotactic response of ATCC10798 [*fliC***(N87K)] and SHU102 [***fliC***(N87K)::***fliC***] cells**

85 (a) The schematic of the tip (capillary) assay. The tip contains a 5-µl buffer containing 1 % (wt/vol) agarose, and the other end was sealed with cray to avoid an effect of oxygen on a chemotactic response. (b) Pseudo-colors of phase-contrast images of ATCC10798 (*top*) and SHU102 cells (*bottom*). In the presence of 1 mM serine, both *E.coli* strains exhibit a chemotactic response and gather near a tip (orange color). Scale bar, 200 μm. (c) Intensity profiles of b. (d) A phase-contrast image of ATCC10798 cells in the presence of 10 mM serine. In the presence of serine, ATCC10798 cells tend to gather and aggregate each other, which was not detected in SHU102 cells. Scale bar, 20 μm.

Supplementary Figure 6 Quantification of rotational rate and structural parameters of flagella of SHU102 under TIRFM

Histograms of the structure and rotation rate of flagella of SHU102. The solid line

97 represents the Gaussian fitting. Peaks and SDs of the rotation rate were 78.9 ± 27.5 Hz (n

98 = 33). The flagellar pitch was 2.3 ± 0.2 µm (n = 30). The pitch angles were 30.2 ± 4.1

99 degrees (n = 30). The helix radius was 0.22 ± 0.04 µm (n = 30).

 Histograms of the structural properties and rotation rate of flagella of *E. coli* HCB1336 (Δ*fliC* strain) carrying the plasmid encoding wild-type *fliC* (pYS10) and *fliC*(N87K) (pSHU61). Solid line represents the Gaussian fitting. Peaks and SDs of the rotation rate were 71.8 ± 14.1 Hz in HCB1336/pYS10 (*top*, n = 41) and 98.4 ± 23.9 Hz in 108 HCB1336/pSHU61 cells (*bottom*, $n = 50$) and. The flagellar pitches were $2.3 \pm 0.1 \mu m$ in HCB1336/pYS10 cells (*top*, n = 40) and 1.1 ± 0.1 μm in HCB1336/pSHU61 cells (*bottom*, 110 $n = 94$). The pitch angles were 30.8 ± 4.1 degree in HCB1336/pYS10 cells (*top*, n = 37) 111 and 39.3 ± 5.5 degree in pSHU61/HCB1336 cells (*bottom*, n = 52). The helix radii were 112 0.22 \pm 0.03 μm in HCB1336/pYS10 cells (*top*, n = 40) and 0.14 \pm 0.03 μm in HCB133/pSHU61 cells (*bottom*, n = 94).

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115 **Supplementary Figure 8 Quantification of motor properties by tethered-cell**

- 116 **assay**
- 117 (a) Switching frequency between *E. coli* strains for 10 sec. The average and SD were 1.15
- 118 \pm 0.60 s⁻¹ in ATCC10798 (n = 99), 1.35 \pm 0.75 s⁻¹ in SHU155 (n = 67), 1.52 \pm 0.84 s⁻¹ in
- 119 W3110 (n = 53), and 1.18 ± 0.56 s⁻¹ in RP437 (n = 49). (b) CW bias (Time_{CW}/Time_{Total}).
- 120 The average and SD were 0.61 ± 0.21 in ATCC10798 (n = 99) and 0.52 ± 0.30 in SHU155
- 121 $(n = 67)$, 0.48 ± 0.27 in W3110 $(n = 53)$, and 0.42 ± 0.23 in RP437 $(n = 49)$. (c) Rotation
- 122 rates. The average and SD were 7.3 ± 1.4 Hz in ATCC10798 (n = 99), 7.3 ± 1.1 Hz in
- 123 SHU155 (n = 67), 7.0 ± 1.3 Hz in W3110 (n = 53), and 7.0 ± 1.2 Hz in RP437 (n = 49).
- 124 ATCC10798 and W3110 data from Figure 3.

Vibrio alginolyticus ATCC17749
Pseudomonas aeruginosa PAO1
Escherichia coli K-12 W3110
Salomonella typhimurium LT2
Bacillus subillis 168
Rhodobacter sphaeroides ATCC 17029
Helicobacter sphaeroides ATCC 17029
Caulobacter re Caulobacter crescentus CB15

Vibrio alginolyticus ATCC17749
Pseudomonas aeruginosa PAO1
Escherichia coli K-12 W3110 Escherichia coli K-12 W3110
Salomonella typhimurium LT2
Bacillus subbilis 168
Rhodobacter sphaeroides ATCC 17029
Helicobacter pylori 26695
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Vibrio alginolyticus ATCC17749 Vibino alginolyticus ATCC17749

Pseudomonas aeruginosa PAO1

Escherichia coli K-12 W3110

Salomonella typhimurium LT2

Salomonella typhimurium LT2

Rhodobacter sphaeroides ATCC 17029

Rhodobacter sphaeroides ATCC 17029

Re ---NLRSDTSAMGGKSYAAEEGKDASWTVGEKTEFK

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 272 174 TSSFTTAAAAKT-

 $\begin{tabular}{c|c|c} \multicolumn{2}{c}{245} \hline \multicolumn{2}{c}{PASSQKVEGDV}\\ \hline 345 \hline \multicolumn{2}{c}{AMTTTVFGYQLNS2FTAY2VSGTGTQASQVFGNASAAQKSSVASVADSTADGAQNAIAVVDNALAA1DAQRADLGAVQNRFNN1DWHNBNNASK} \\ \hline 345 \hline \multicolumn{2}{c}{AMTTTVTGYVQLNS2FTAY2VSGTGTQASQVFGNASAAQKSSVASVADSTADGAQNAIAVVDNALAA1DAQRADLGAVQNRFKNT1DMLTN1SSRANAR1X0ZT7X7X$

 $333 {\tt SRIKPTDVAKETTAMTKSQIDQQASTSILAQAKQSPSANLSLIG-445 {\tt SRIKPTDPABETAALSKNQUDQAGTAILAQANQVPQQVLSLIG-454 {\tt SRIQDADYATEVSMMSRAQIDQQAGISVLAAQANQVPQQVLSLIG-462 {\tt SRIEDSDYATENSMMSRAQIDQQAGTQVDQAWLSLLIG-471 {\tt SQTADVDAPEABSTNILASQGLSQASTAMAQANOSKQNULSLLIG-471 {\tt SQTAD}DPAAESTNILASQIT.SQASTAMIAQANOSKQNUVLSLIG-471 {\tt SQTAD}DAPALSCHIATIATIAG$ 230 GNLVDADLAKESAKLQSLQTKQQLGVQALSIANQSSSSILSLFR-

Supplementary Figure 9 Structure and sequence of flagellin

 (a) Subunit structure of L-type flagellar subunit (PDB: 3A5X). The important residues for a curly filament are shown. (b) Side views of L-type flagellar filament. The number of protofilaments is shown. *Inset*: Top view of filaments. (c) L-type (*left,* PDB: 3A5X) 130 and R-type (*right*, PDB: 1UCU) straight filaments of *S. typhimurium* are shown ^{[3,](#page-16-0)[4](#page-16-1)}. Hydrogen bonds interaction (black) between S0 (green) and S5 subunits (blue). E84, N87, E122, T130 of the S0 subunit are colored in yellow, red, orange, and white, respectively; T438 and N439 of the S5 subunit are colored in cyan and pink, respectively. (d) Alignments of the flagellin amino acid sequence from typical bacteria. The red star represents N87 residue; blue stars indicate E84, E122, T130, T438, N439 residues. Images were generated using PyMOL 2.4 [\(https://pymol.org/2/\)](https://pymol.org/2/).

Supplementary Figure 10 Growth curve in *Escherichia coli* **K-12 ATCC10798 and W3110**

 The single colony was scrutinized by the tip, resuspended into 50-ml LB medium, and 142 then cultured at 37°C with shaking. The absorbance of cells at 600 nm were periodically measured with 60-min intervals. During the exponential phase, we fitted the data as the 144 following equation: $f(t) = Ax \frac{t}{2 \tan \theta}$, where *A* the constant and *tau* the doubling time. The doubling time was estimated to be ~24 min in ATCC10798 (red) and 30 min in W3110 (blue). The experiments were performed two times.

149 **Supplementary Table 1**

150 Structural parameters and kinematics of the cell body and flagella in *Escherichia coli*.

- 151 Values were directly measured by either a transmission electron microscopy (EM) or
- 152 optical microscopy (OM).

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Supplementary Table 2. Strains and plasmids

157 **Supplementary Table 3. Primers**

Captions for Supplementary Videos

- Supplementary Video 1
- Swimming motility of *E. coli* K-12 ATCC10798 (*left*) and *E. coli* K-12 W3110 (*right*)
- observed under a phase-contrast microscope. ATCC10798 cells show 180°-
- reversals against to original swimming direction with the cell body rolling, whereas
- 164 W3110 cells swim with the run and tumble movements. Scale bar, 20 um.
- Supplementary Video 2
- Swimming motility of ATCC10798 (*left*) and W3110 (*right*) under a conventional
- fluorescent microscope. Scale bar, 20 μm.
- Supplementary Video 3
- High-speed imaging of flagellar rotation of ATCC10798 (*left*) and W3110 (*right*)
- 170 using an EMCCD camera under a fluorescent microscope. Scale bar, 5 um.
- Supplementary Video 4
- Flagellar rotation of ATCC10798 under TIRFM. Scale bar, 4 μm.
- Supplementary Video 5
- Flagellar rotation of W3110 under TIRFM. Scale bar, 5 μm.
- Supplementary Video 6
- Real-time imaging of a polymorphic flagellar change under TIRFM. A rotational
- direction was changed at 0.11 sec; subsequently, a normal left-handed filament
- was transformed into the right-handed semi-coiled filament. Scale bar, 2 μm.
- Supplementary Video 7
- Real-time imaging of a polymorphic flagellar change under TIRFM. A rotational
- direction was changed at 0.17 sec; subsequently, a right-handed curly filament
- was transformed into the normal left-handed filament. Scale bar, 2 μm.
- Supplementary Video 8
- Swimming motility of SHU102 (*left*), HCB1336/pYS10 (*middle*)*,* and HCB1336/pSHU61 cells (*right*) under a phase-contrast microscope. Scale bar, 50 μm.

- Supplementary Video 9
- Swimming motility of SHU102 cells under a conventional fluorescent microscope.
- Scale bar, 20 μm.
- Supplementary Video 10

Chemotactic response of ATCC10798 (*top*) and SHU102 cells (*bottom*) under a

 phase-contrast microscope. For the first 10 sec, cells swim in the absence of 194 serine. For the last 10 sec, both cells swim in the presence of 1 mM serine. Scale

- bar, 200 μm.
- Supplementary Video 11

 Flagellar rotation of SHU102 (*left*), HCB1336/pYS10 (*middle*)*,* and HCB1336/pSHU61 cells (*right*) under TIRFM. Scale bar, 2 μm.

- Supplementary Video 12
- Swimming motility of ATCC10798 (*left*) and W3110 (*right*) on a 0.2 % agarose
- pad. ATCC10798 cells cannot migrate after stuck, whereas W3110 cells keep moving with 180°-reversals to escape from a stuck. Scale bar, 50 μm.
- Supplementary Video 13
- Swimming motility of ATCC10798 (*left*) and W3110 (*right*) in the presence of 15 %
- ficoll. Scale bar, 20 μm.

Supplementary References

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