Supplementary Material for

The effect of cell aspect ratio on swarming bacteria

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Growth protocol and observation

Bacillus subtilis is a Gram positive, rod-shaped, flagellated bacterial species, used as a model system in many quantitative swarming experiments [6-8, 11-13, 19, 22-23, 25]. Nine different variants of *B. subtilis* 3610 were tested, all with the same width (~0.8 μm) but varying lengths. The cells were grown on agar plates; multilayer swarming colonies were obtained by growing the colonies on 1 g/l peptone (Becton, Dickinson) and 0.5% agar. Plates were filled with 20 ml of molten agar and aged for 24 h in the lab (20ºC and 45% RH) prior to inoculation. The cells were incubated at 30ºC and 95% RH for about 5 h. *B. subtilis* is normally kept at -80ºC in 50% glycerol stocks and grown overnight in LB broth at 30ºC and shaking (200 RPM) prior to plate inoculation (5-μl at the center of each plate; OD_{650}=1, corresponding to approximately 10^7 cells/ml).

All mutants were obtained from the same lab (Daniel B. Kearns, Indiana; see also [20]). Table 1 lists the strain name and the mean aspect ratio with the standard deviation. The mean was obtained from 200 randomly chosen cells in the active swarm – cells were pulled off the swarm by placing a small water drop on the colony edge, then immediately measured. In most cases the large variety of cell-lengths in a specific sample is due to proliferation and cell division thus the mean cell length does not have a Gaussian distribution (size is limited by the length of a single cell and the length of twice its size).

An optical microscope (Zeiss Axio Imager Z2; 20X, 40X and 63X LD-Phase contrast lenses), equipped with a camera (GX 1050, Allied Vision Technologies) was used to capture the microscopic motion at 100 f/s and 1024×1024 pixels. Each experiment from a single plate lasted 30 s (3000 frames), and 10 independent experiments were performed for each strain. Recorded movies were converted to a sequence of single-frame images. Following standard pre-processing for noise reduction, the optical flow (OF) between each two consecutive frames was obtained using Matlab. Vector fields were reduced to a 64×64 grid by simple averaging, generating an approximated velocity and a vorticity fields (see additional information in [7, 11-12]).

Control experiments

In order to verify that the differences in the swarming dynamics statistics are only due to aspect ratio, several control tests were performed. This is important because the 8 mutations were generated in different ways, genetically affecting specific genes to form short or long cells. These tests guaranty that on the single cell level, cell motility was not affected.

Figure S1 shows that the swimming ability of the bacteria in sparse (10^4 cells/ml) liquid suspensions, where interactions between cells are negligible and there is no interaction with a surface, is the same for all strains. Both WT and all 8 mutants swam in the well-known 'run-and-tumble' mode with approximately similar speeds (~30 μm/s) during 'runs'.

Figure S2 shows that the ability of all strains to colonize swarm plates is fairly constant. All strains showed a macroscopic swarm pattern and covered a standard Petri-dish (8.8 cm) within a few hours, moving the colony front at approximately the same speed (~10 mm/h).

Transmission electron microscopy (TEM) observations of the cells (see example in **Fig. S3**) showed several flagella stemming from each cell, independently on the strain. No significant difference in the average flagellar density across the surface, structure and location on the membrane could be observed. For flagellar observations, a FEI Tecnai transmission electron microscope (TEM), operated at 120 kV, was used. The rod-shaped cells were collected from the agar using different methods and placed on 400-mesh copper carbon grids (Electron Microscopy Sciences). The best results were obtained by gently stamping the grids on the live colony at the region of interest for 1 s and then lifting the grid. The sample was immediately stained with 0.5% (wt/vol) uranyl acetate for 10 s, which fixes the cells and flagella in the state they were in at the time of contact with the grid.

The contact angle of drops of supernatant taken from overnight cultures and deposited on flat smooth surfaces is an indication for the amount of surfactants secreted by cells. **Figure S4** shows that this angle varied significantly between mutants with no clear dependence on cell length (Fig. S4A) or microscopic speed on the agar (Fig. S4B). However, we found little correlation between the measured contact angle and the colony expansion speed (Fig. S4C) indicating the reliability of our control measurements (note that the surfactant experiments were performed on supernatant taken from swimming cells where swarming motility is not expressed).

Growth curves for the nine strains were obtained. Each strain was grown overnight from a plate stock (with the appropriate antibiotics) in a separate 50 ml tube in fresh LB broth (with no antibiotics) at 30 ºC and shaking (200 RPM) (10 ml in each tube). The overnight cultures were diluted (1/100 fold) and regrown at same conditions. The number of CFU grown on 1.5% agar LB plates from these suspensions (after appropriate culture dilution) for each strain was counted; this was repeated during few hours from first dilution. The experiments were done few times so that the optical density was adjusted in all strains to yield same number of CFU in the starting culture. **Figure S5** shows a summary of these measurements. The data shows that the doubling time is similar in all strains (approximately 55 min) with very small variations in the duration of the lag phase.

To measure the number density and mass density as a function of aspect ratio, cells were taken from the swarm edge. A sterile 1000 μl tip was inserted to each colony and immediately

removed. Each tip was inserted into a small tube filled with 100 µl of LB broth, letting the cells migrate to the volume. The tip scratched a circular region with an approximated diameter of 300 µm. Each 100 µl of LB broth with cells were diluted 10, 100 and 1000 fold and inoculated on 2% agar LB plates with Drigalski sticks. After a few hours of incubation, colonies were counted indicating the number of CFUs in the region from which the cells were taken. Colony height was estimated to be 3-layers based on phase-contrast microscopic observations. Mass density was estimated based on a cylindrical cell shape with diameter of 0.8 µm (**See Fig. S6**).

Figure S7 shows a linear trend in the standard deviation of cell aspect ratio for each strain as a function of mean cell aspect ratio. This indicates that relatively small, or large, deviations in cell length within a strain are not affecting the collective dynamics.

Overall, these results indicated that all mutants have similar swimming and swarming capabilities with no apparent or immediate motility defects.

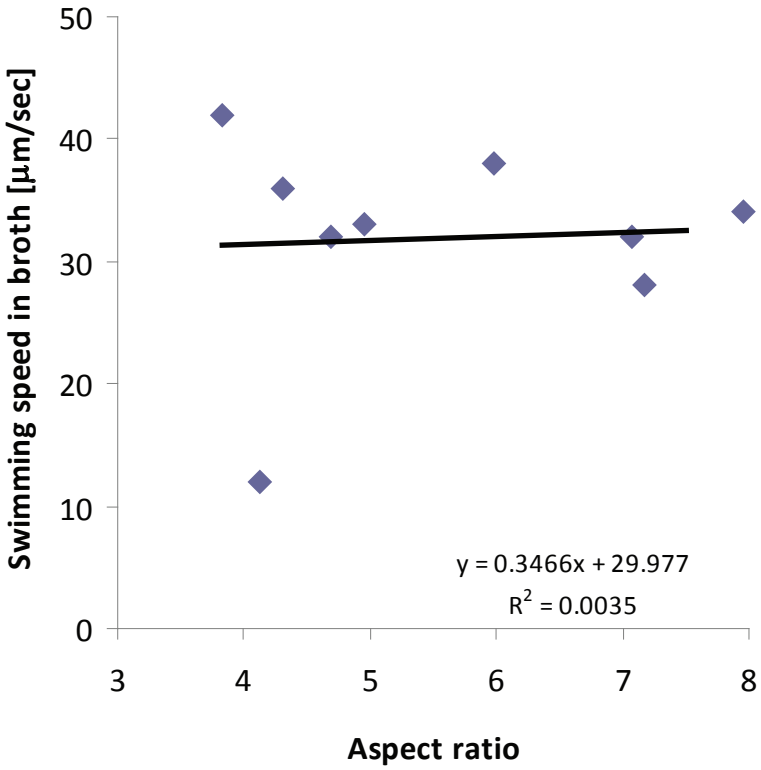
Swimming speed in broth [µm/sec]
50
40
30
20
10
0
3
4
5
6
7
8
Aspect ratio
y = 0.3466x + 29.977
R2 = 0.0035

Fig. S1

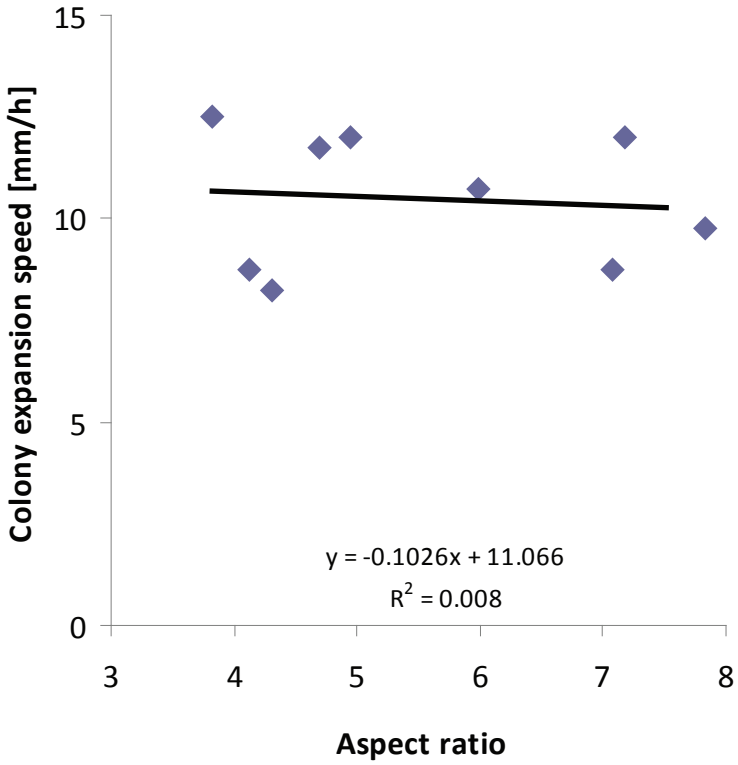
15
10
5
0
Colony expansion speed [mm/h]
y = -0.1026x + 11.066
R2 = 0.008
3
4
5
6
7
8
Aspect ratio

Fig. S2

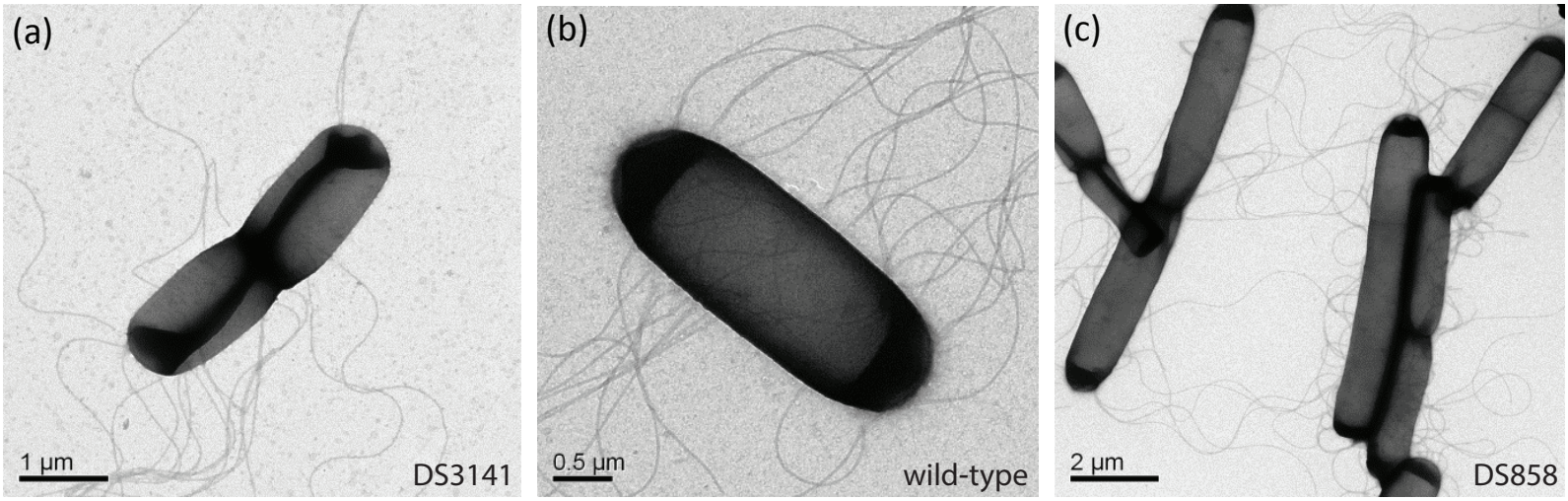

Fig. S3

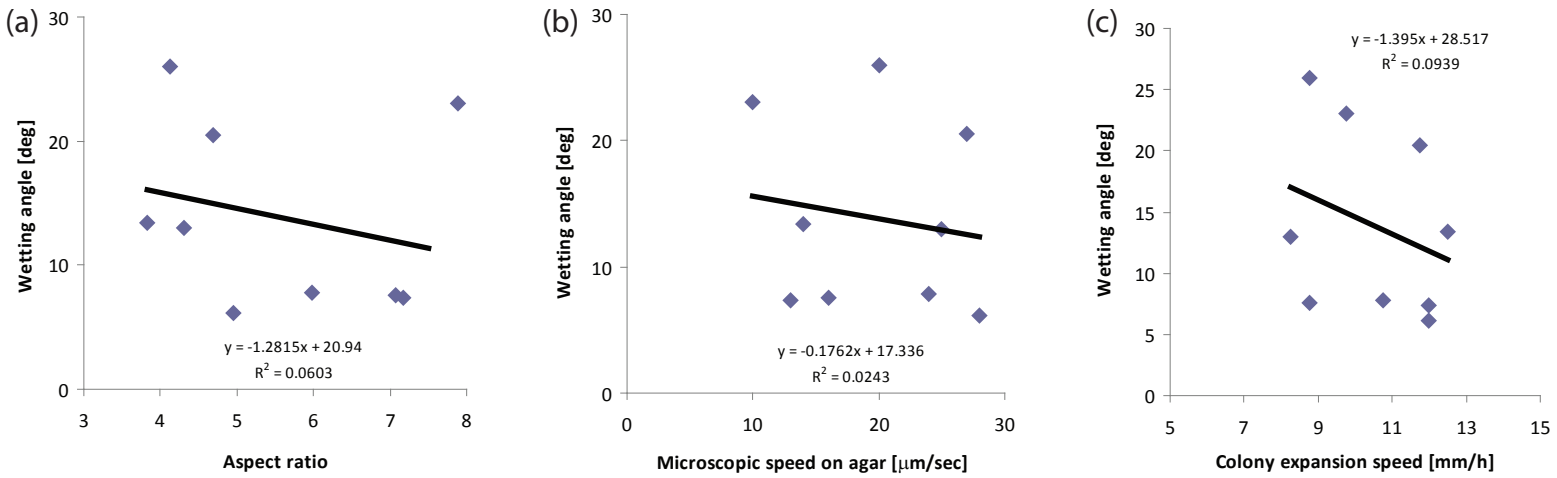
(a)
Wetting angle [deg]
Aspect ratio
y = -1.2815x + 20.94
R2 = 0.0603
(b)
Wetting angle [deg]
Microscopic speed on agar [μm/sec]
y = -0.1762x + 17.336
R2 = 0.0243
(c)
Wetting angle [deg]
Colony expansion speed [mm/h]
y = -1.395x + 28.517
R2 = 0.0939

Fig. S4

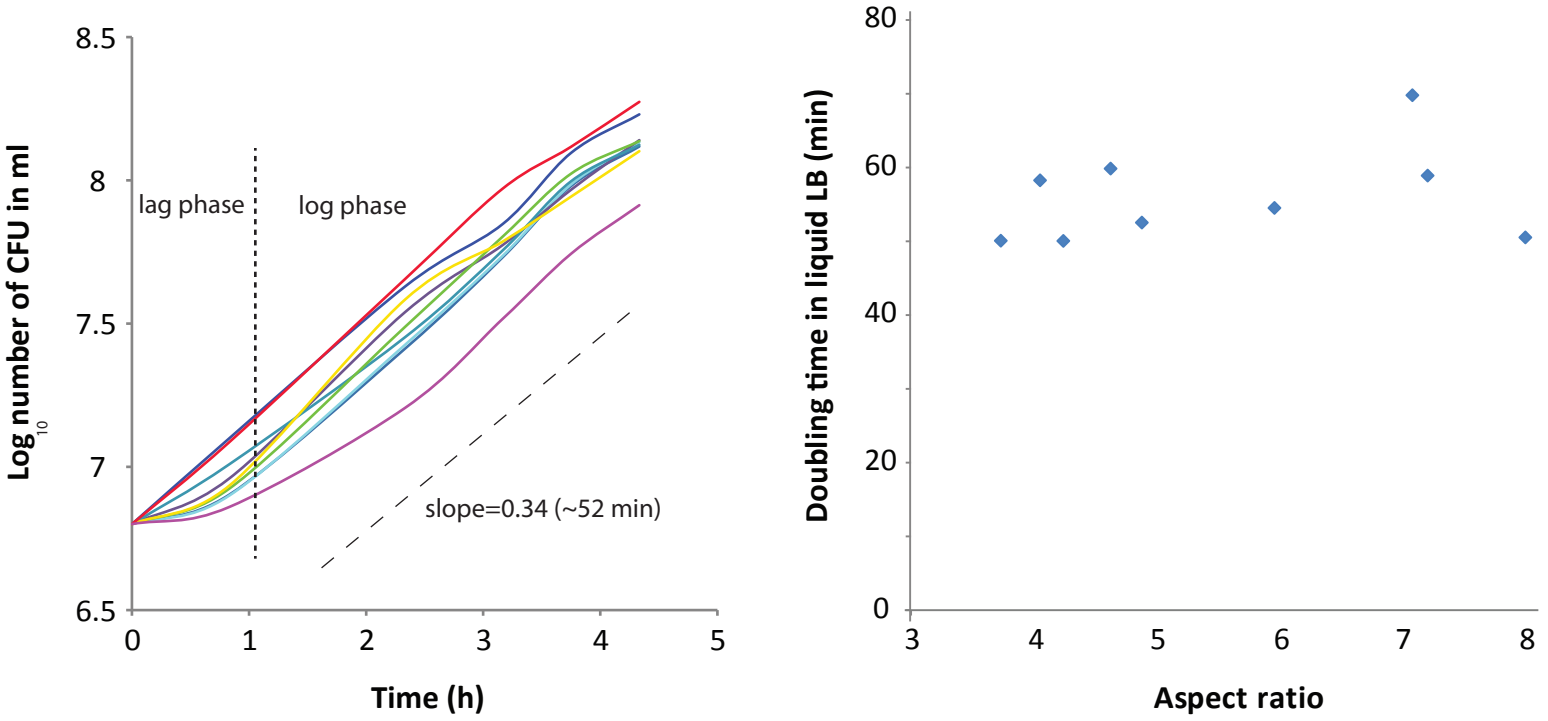
8.5
8
7.5
7
6.5
Log10 number of CFU in ml
lag phase
log phase
slope=0.34 (~52 min)
0
1
2
3
4
5
Time (h)
80
60
40
20
0
Doubling time in liquid LB (min)
3
4
5
6
7
8
Aspect ratio

Fig. S5

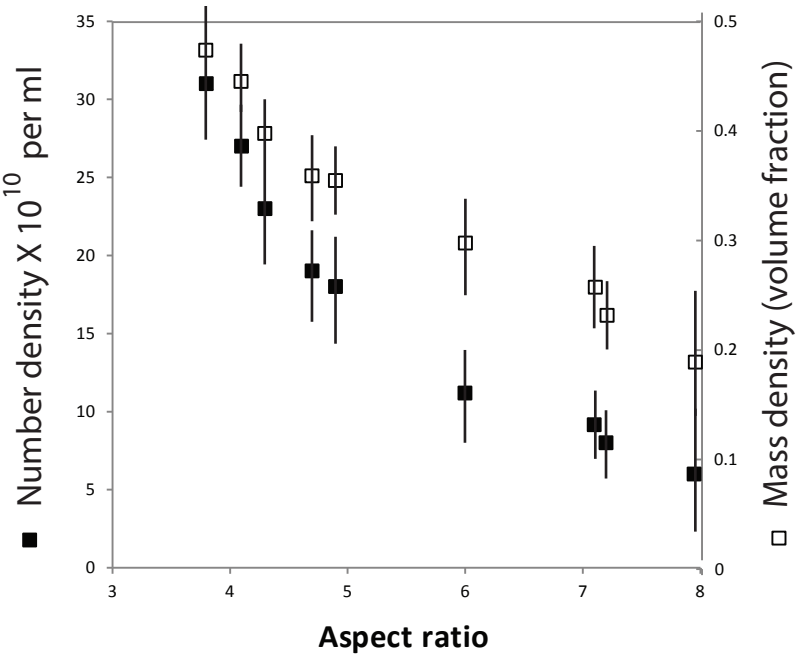
Number density X 10^10 per ml
Mass density (volume fraction)
Aspect ratio
0
5
10
15
20
25
30
35
0
0.1
0.2
0.3
0.4
0.5
3
4
5
6
7
8

Fig. S6

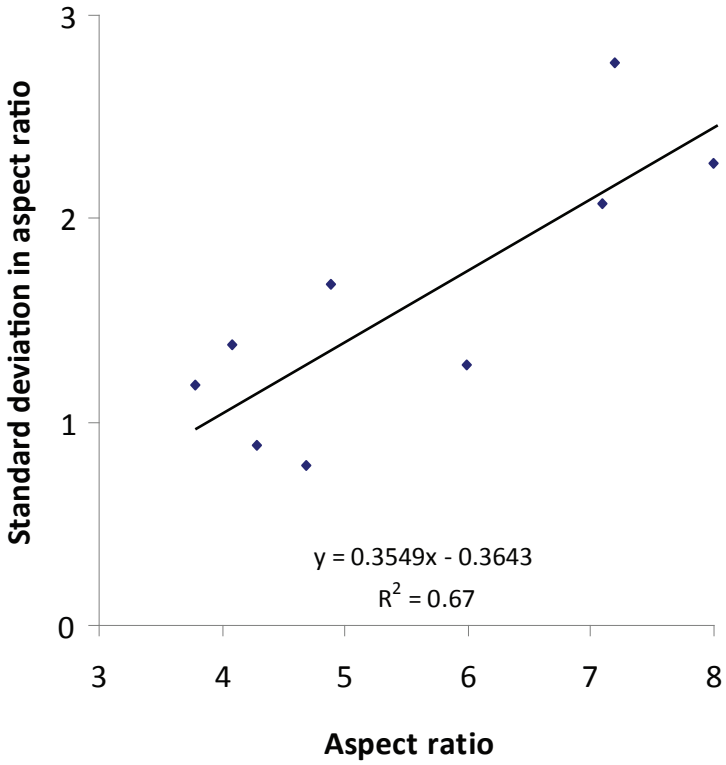
Standard deviation in aspect ratio
Aspect ratio
y = 0.3549x - 0.3643
R2 = 0.67
0
1
2
3
3
4
5
6
7
8

Fig. S7