

Supplementary Information (SI)

The single-cell chemostat: an agarose-based, microfluidic device for high-throughput, single-cell studies of bacteria and bacterial communities

Supplementary Discussion

Production-Diffusion Model for the Concentration Gradient Created by a Linear Colony

To better understand the proximity-dependent growth rates observed in Figure 5, we consider here a simple model for the metabolite concentration profile produced by a linear colony. We assume, for simplicity, that the production rate per unit length of the colony—the number of metabolite molecules excreted per unit time per unit length— η , is uniform. Moreover, we neglect the complicated effects of buffer flow through the gutters and assume only that removal of metabolite by buffer flow allows the system to relax to a steady state. Finally, we neglect the reflecting boundary condition of the glass coverslip. To first order, the effect of metabolite removal by the gutters and the glass coverslip will be to simply rescale the effective production rate of the colony, η . More detailed calculations, beyond the scope of current work, would be required to extract this parameter from our data.

At steady-state, the concentration, $c'(x, y)$, of the metabolite excreted by an infinitesimal length of a line source, dl , centered at the origin is governed by

$$D\nabla^2 c'(x, y) = -\eta dl \delta(\vec{r})$$

where D is the diffusion constant for the excreted metabolite. The solution of this expression is

$$c'(x, y) = \frac{\eta}{4\pi D} \frac{dl}{\sqrt{x^2 + y^2}}.$$

The total concentration produced by a linear colony of length L centered at the origin and oriented along the x-axis is the integral over all infinitesimal line segments:

$$c(x, y) = \frac{\eta}{4\pi D} \int_{-L/2}^{L/2} \frac{1}{\sqrt{(x-l)^2 + y^2}} dl = \frac{\eta}{4\pi D} \left[\sinh^{-1} \left(\frac{L-2x}{2y} \right) - \sinh^{-1} \left(-\frac{L+2x}{2y} \right) \right]$$

where l is the position of an infinitesimal point charge along the linear colony.

To further simplify our analysis, we approximate the concentration observed by a linear colony of the complementary auxotroph at some distance y from the metabolite source by the concentration at the center of that colony, i.e. $x = 0$. Thus,

$$c(0, y) = \frac{\eta}{2\pi D} \left[\operatorname{csch}^{-1} \left(\frac{2y}{L} \right) \right].$$

For all of the complementation experiments reported here, the observed growth rates for the auxotrophs are smaller than that observed when the needed amino acids are added, indicating that the excreted metabolite is limiting for growth. Thus, we expect the growth rate of the auxotroph to be approximately linear in the concentration of the needed metabolite, i.e. $g(y) = \gamma c(y)$. In addition, because we do not observe colonies separated by distances larger than our field of view, 100 μm , we expect to observe an average background growth rate, g_0 , that is due to the average metabolite concentration produced by these unobserved, distant colonies. Thus, under our simplifying assumptions, we expect a proximity-dependent growth rate of

$$g(y) = a \left[\operatorname{csch}^{-1} \left(\frac{2y}{L} \right) \right] + g_0$$

where the coefficient contains the production rate, diffusion constant, and proportionality constant between growth rate and metabolite concentration: $a = \gamma \frac{\eta}{2\pi D}$.

Supplementary Figures

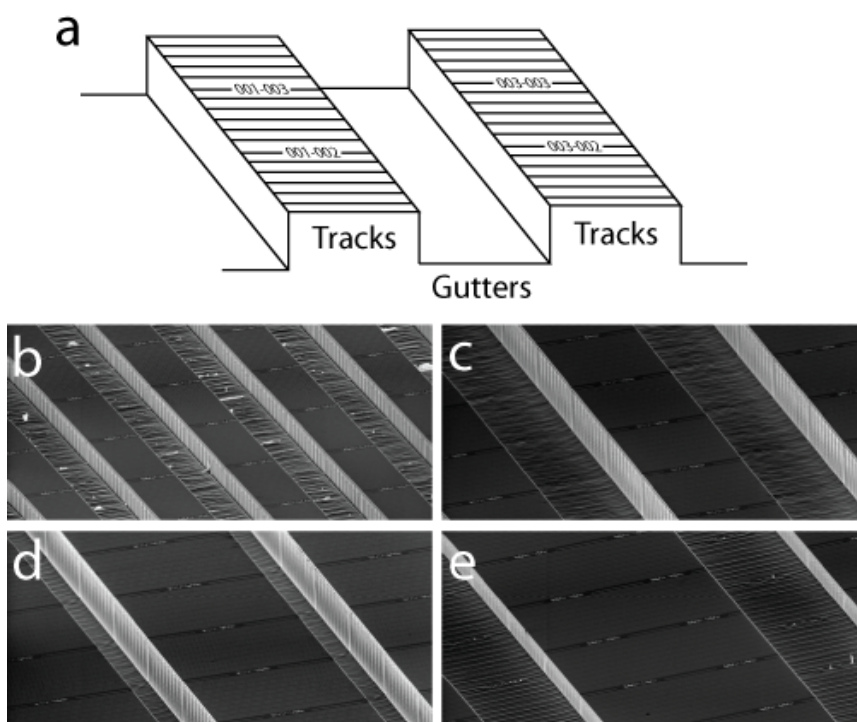


Figure S1: Views of different silicon masters. (a) Schematic diagram of the angled view of the silicon master pictured in this figure. (b)-(e) SEM images of silicon masters with (b) 50- μm long tracks and 50- μm wide by 20- μm deep gutters, (c) 100- μm long tracks with 100- μm wide by 40- μm deep gutters, (d) 150- μm long tracks with 50- μm wide by 40- μm deep gutters, and (e) 200- μm long tracks with 100- μm wide by 20- μm deep gutters. The 50- μm long tracks were printed with shallower gutters because agarose features with aspect ratios larger than ~ 0.5 (height to width) are easily damaged. The same shallow gutters were used with the 200- μm long tracks because the 50- μm and 200- μm long tracks were fabricated on the same silicon wafer. Images were collected with a 65 degree tilt from perpendicular, and all images share the same scale. *E. coli* has been successfully cultivated in rich defined medium on patterned agarose with tracks 1.5- μm deep, 500 – 700-nm wide, and with each of the lengths pictured here (data not shown).

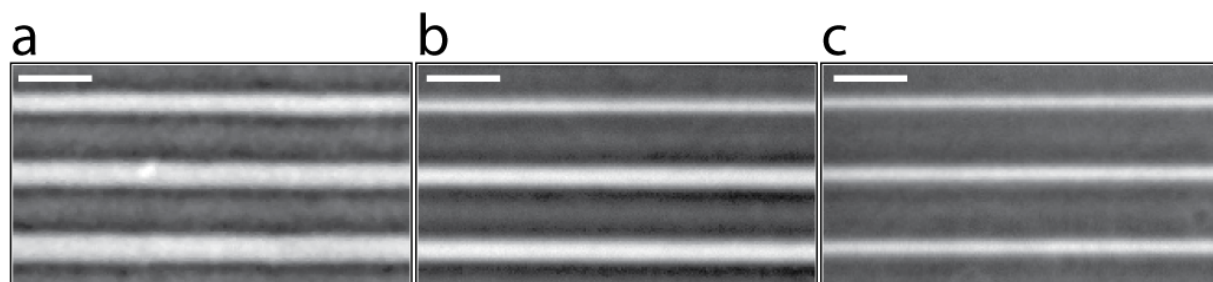


Figure S2: Patterned tracks of different widths. (a)-(c) Phase contrast images of patterned agarose gels (5% w/v) created with tracks decreasing in width from 1.0 μm (bottom of panel a) to 300 nm (top of panel c.) Gels created from silicon masters that (a) were not coated with SiO_2 or are coated with layers (b) 200 nm or (c) 400 nm thick. Tracks increase in width by approximately 100 nm from top to bottom in each image. Scale bars correspond to 2 μm , the smallest width previously printed into agarose. All pictured tracks are 1.5- μm deep though we have also printed tracks of the same width with depths of 1.25 μm and 1.0 μm (data not shown.)

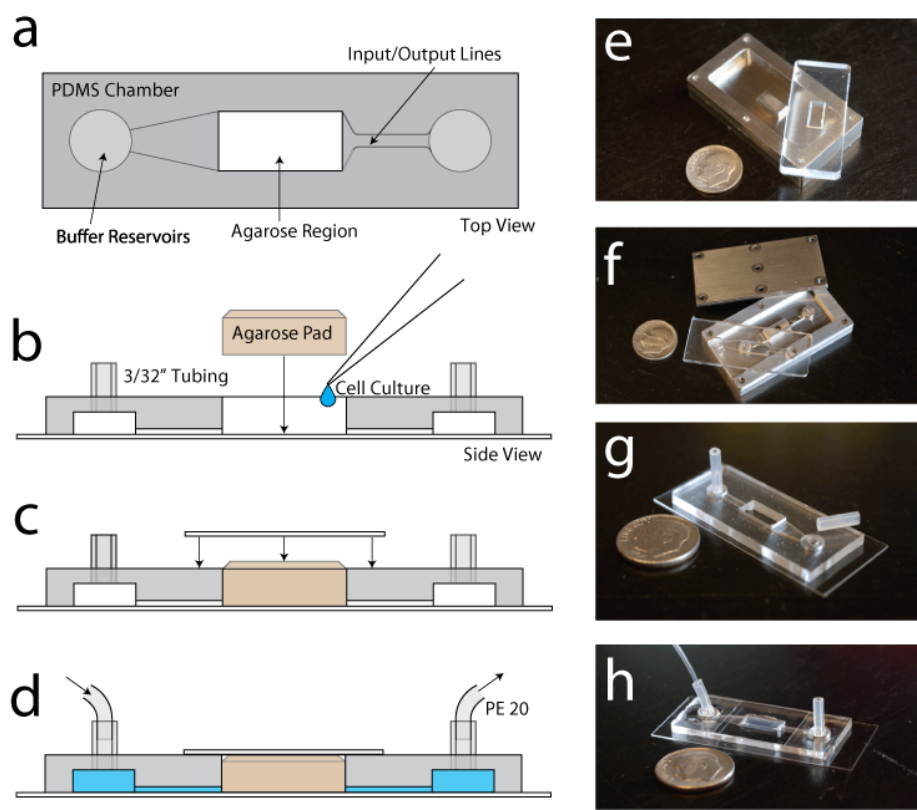


Figure S3: Experiment assembly. (a) Top view of the PDMS sidewall. (b) Side view of a partially assembled chamber. The bottom cover glass and the Tygon tubing have been added to create a chamber ready for cells and the printed pad. Cells are added to the chamber and confined between a patterned agarose pad and a glass cover slip. (c) Once the cells and the pad are loaded, an additional cover glass compresses and seals the device. (d) PE tubing is then used to introduce and remove buffer. (e) A PDMS gel mold and the stainless steel mold used to fabricate it. (f) A PMDS sidewall with the stainless steel mold used to create it. (g) A partially assembled chamber with Tygon tubing. (h) A fully assembled chamber with top cover slip and pad.

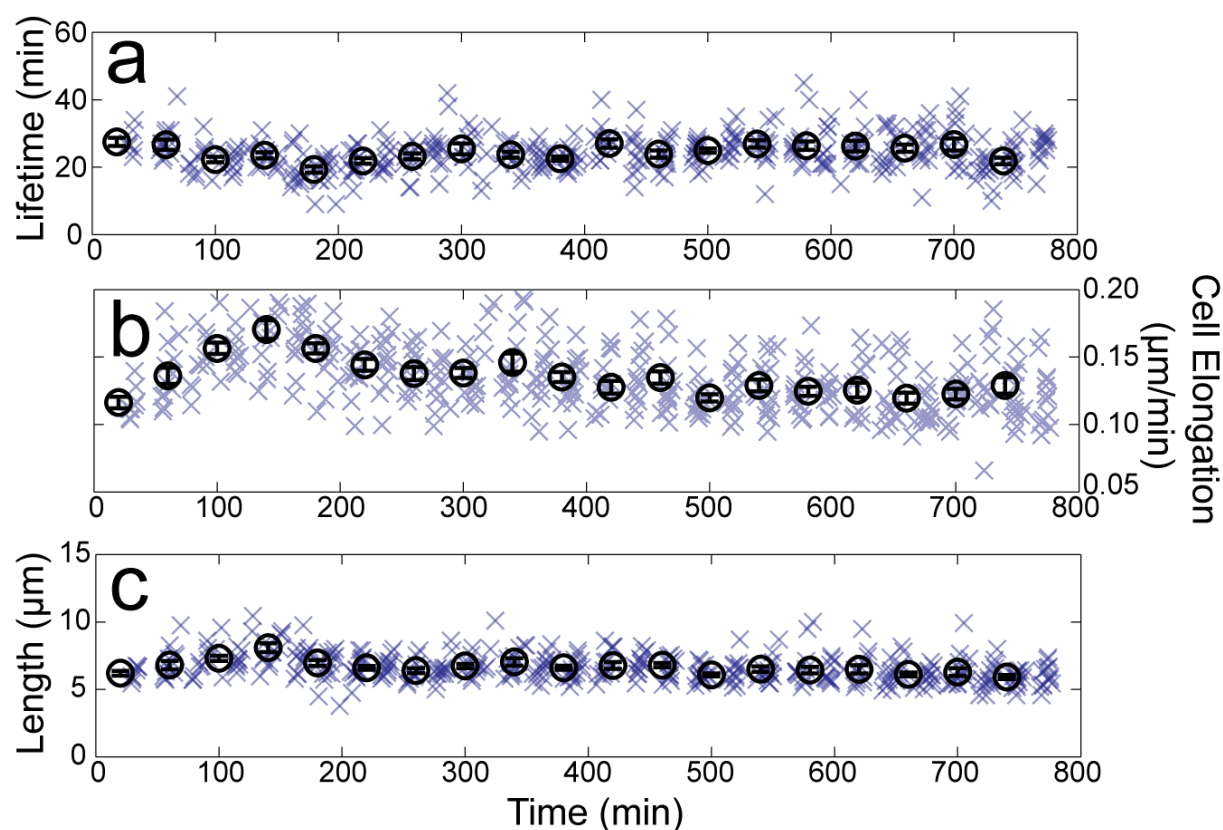


Figure S4: Growth properties of individual cells reveal a chemostatic environment. (a) Lifetime (or cell division time), (b) average cell elongation rate, and (c) length at division for all cells growing in the colony presented in Figure 2 and for which the initial and final division was observed. Average growth properties for individual cells (purple crosses) are plotted at times corresponding to the middle of the life of each cell. Averages (black circles) are computed over all cells within a 40-minute window, and error bars represent the standard error of the mean. Fluctuations in the average growth properties do not correlate between adjacent colonies (data not shown), revealing that these fluctuations are of biological origin.

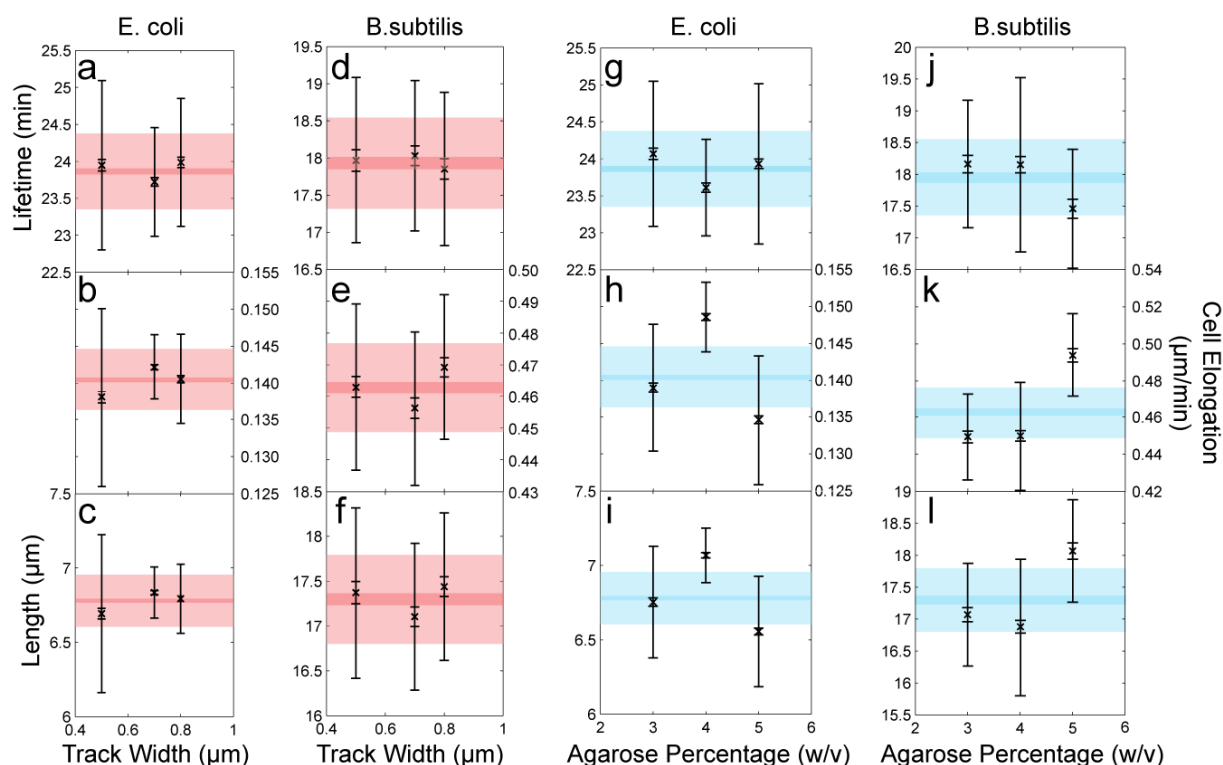


Figure S5: Growth does not vary with track width or agarose percentage. (a) Average lifetime, (b) cell elongation rate, and (c) length at division for *E. coli* growing in tracks of different widths. (d)-(f) The same growth properties for *B. subtilis* as a function of track width. All experiments were conducted with 1.5-μm deep tracks. In the rich medium of these experiments, *E. coli* has a diameter of ~0.8 μm, and *B. subtilis* has a diameter of ~1.0 μm; thus, most tracks are narrower but deeper than cells. (g-i) *E. coli* and (j-l) *B. subtilis* growth properties as a function of agarose percentage (w/v). Increasing the agarose percentage produces gels that are stiffer and, thus, exert more pressure on the cells. Black symbols represent the mean of all measured cells. The smaller error bars represent the standard error of the mean with respect to the number of cells while the larger error bars represent the standard error with respect to the number of clonal replicates, i.e. the number of colonies. The shaded regions are centered on the global mean, and the inner and outer regions correspond to the two standard errors of this global mean. No systematic trend with track width or agarose percentage is observed for any of the growth properties, indicating that the pressure applied by the tracks does not perturb the growth of either of these bacteria.

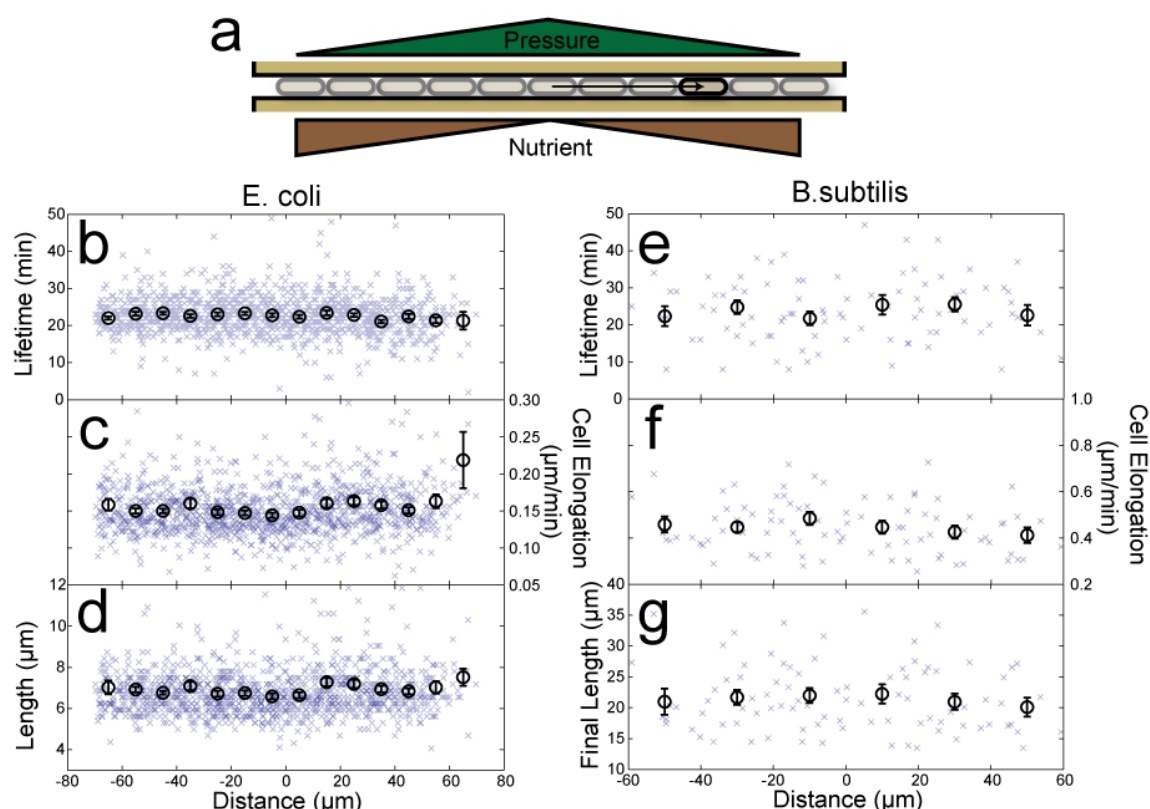


Figure S6: Growth does not vary with position in track. (a) Cartoon representation of the potential perturbations experienced by cells based on their position within the tracks. Near the center of the colonies, cells must generate the pressure required to push cells out of the colony. In addition, cells near the center are further from the nutrient source in the gutters and potentially observe a different nutrient environment. Both perturbations would predict that cells towards the center of the colony would grow more slowly than cells near the ends of the tracks. (b) Lifetime, (c) average cell elongation rate, and (d) length at division for *E. coli* growing in a 200-μm long colony as a function of distance from the average position of the source during the life of each cell. 200-μm long tracks were used to accentuate any potential position dependence. Distance is defined from the central stationary position in the colony (Fig. 2e), which typically is within ~10 μm of the geometric center of the colony. (e) Lifetime, (f) average cell elongation rate, and (g) length at division for *B. subtilis* growing in a 200-μm-long colony. Individual crosses represent the values for distinct cells while the circles represent the average for all cells in a 10-μm (*E. coli*) or 20-μm (*B. subtilis*) window. Error bars represent the standard error of the mean. No systematic trend as a function of length is observed, indicating that i) the pressure required to push out daughter cells does not perturb growth and ii) nutrient is supplied uniformly to all cells, even cells 100 μm from the gutters.

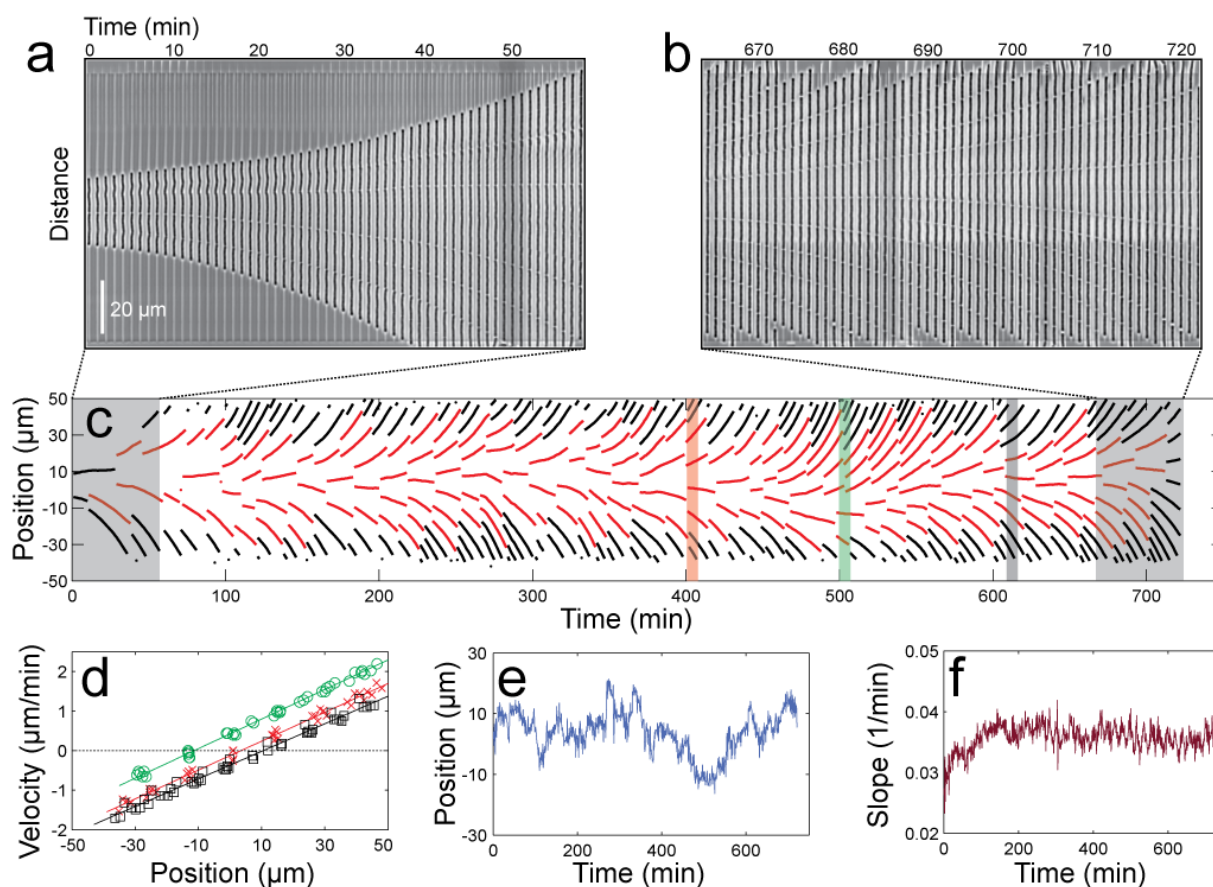


Figure S7: Growth dynamics of *B. subtilis*. (a) A kymograph-like image of a single *B. subtilis* colony growing at 35°C in LB showing the first hour of growth after plating on the structured agarose pad. (b) A kymograph-like image of the same colony 12 hours later, showing that the morphology of the cells is identical to the morphology observed at the beginning of the measurement. (c) The position of each cell within the colony as a function of time. Red lines correspond to cells for which the initial and final division have been observed, and black lines correspond to cells for which one of the divisions was not observed. (d) Center velocity as a function of position for cells at different times. Data are collected from 5-minute windows indicated by the similarly colored regions pictured in (b). Solid lines represent linear fits. (e) Stationary position within the colony as a function of time. Motion of this position indicates that cells can move freely within the track. (f) Expansion rate of the colony (slope of lines in panel (d)) as a function of time. After an initial response to being transferred to the device, the *B. subtilis* colony grows at a constant rate. Nearly 40 generations were observed under chemostatic conditions in this measurement.

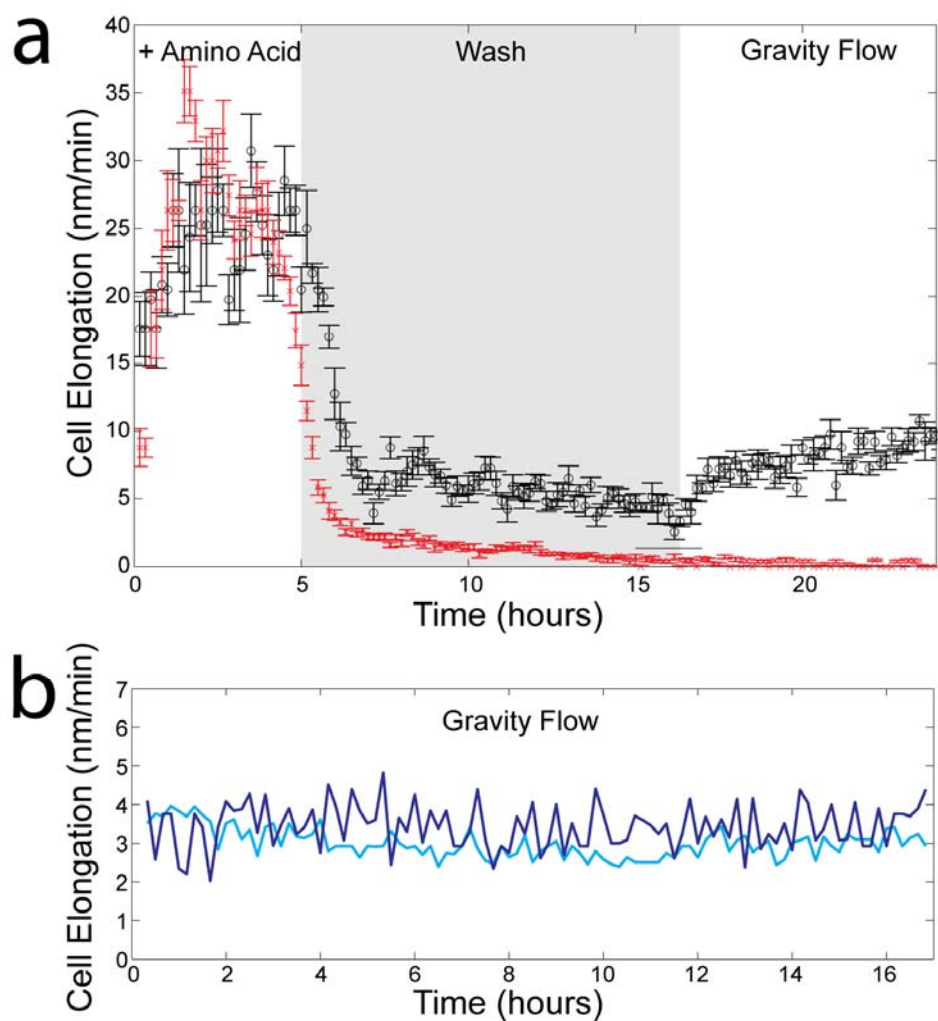


Figure S8: Preparation and chemostatic growth of a microbial community. (a) The median cell elongation rate for all cells during the preparation of a microbial community. Black corresponds to the growth of Δ ilvE when equal numbers of Δ argC are present while red corresponds to the control experiment when Δ ilvE is alone. Error bars correspond to the standard error of the mean. During the first 5 hours, the presence of amino acids dissolved into the gel (10 μ M isoleucine, leucine, valine, and arginine) allows the auxotrophs to grow and fill the tracks. There is no flow during this period. Once growth has established the community, residual amino acid is removed by flowing minimal medium with glucose but without amino acids through the gutters at 10 μ L/min. 12 hours of wash is required to reduce the growth of each auxotroph alone to background levels. After the wash step, the flow rate is changed, and the communication in the colony is allowed to equilibrate for 3 hours. A variety of flow rates have been used during this period; depicted here is gravity flow, in which a pressure difference between the buffer reservoir and the waste reservoir, due to a height difference, produces a slow flow (< 1 μ L/min) that clears the gutters and provides nutrient. (b) The median cell elongation rate for Δ ilvE (cyan) and Δ argC (blue) cells for 17 hours after the completion of the wash step in a community composed of 1/10 Δ argC.

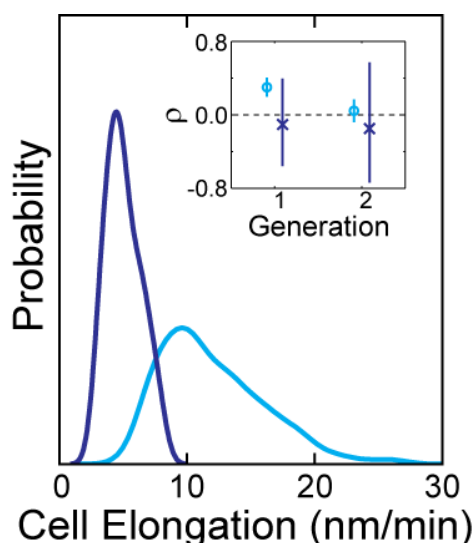


Figure S9: Growth within the community is homogeneous. Probability distributions for the average cell elongation rate of individual $\Delta ilvE$ (cyan) and $\Delta argC$ (blue) cells calculated with kernel density estimation and the optimal kernel. Data are taken from a community of equal numbers of the two strains. Inset: Spearman correlation coefficient for the inter-generational correlation of the average elongation rate between mothers and daughters (generation 1) and mothers and grand-daughters (generation 2). Bars correspond to 95% confidence intervals. To correct for colony-to-colony variations in growth rate, cells were ranked within colonies, and then the Pearson correlation coefficient was calculated for these ranks.

Supplementary Movie Captions

Movie S1: Growth of *E. coli* in rich medium. Phase contrast time-lapse movie of *E. coli*, MG1655, growing in tracks 1.5- μm deep, 0.6 – 0.8- μm wide, and 100- μm long in 5% w/v agarose with rich defined medium at 35C. The frame rate is 1 per minute, and the entire movie runs for 4 hours.

Movie S2: Growth of *E. coli* in minimal medium with glucose. Phase contrast time-lapse movie of *E. coli*, MG1655, growing in tracks 1.5- μm deep, 0.6 – 0.8- μm wide, and 100- μm long in 4% w/v agarose with minimal defined medium and glucose at 35C. The frame rate is 1 per minute, and the movie runs for 4 hours.

Movie S3: Growth of *E. coli* in minimal medium with glycerol. Phase contrast time-lapse movie of *E. coli*, MG1655, growing in tracks 1.5- μm deep, 0.6 – 0.8- μm wide, and 100- μm long in 4% w/v agarose with minimal defined medium and glycerol at 35C. The frame rate is 1 per 4 minutes, and the movie runs for 4 hours.

Movie S4: Growth of *B. subtilis* in rich medium. Phase contrast time-lapse movie of *B. subtilis*, 3610, growing in tracks 1.5- μm deep, 0.6 – 0.8- μm wide, and 100- μm long in 5% w/v agarose with LB at 35C. The frame rate is 1 per minute, and the movie runs for 4 hours.

Movie S5: Growth of *E. faecalis* in rich medium. Phase contrast time-lapse movie of *E. faecalis*, E1sol, growing in tracks 1.5- μm deep, 0.6 – 0.8- μm wide, and 50- μm long in 4% w/v agarose with BHI at 35C. The frame rate is 1 per minute, and the movie runs for 3 hours.

Movie S6: Community growth of *E. coli* prototroph and ΔilvE . Phase contrast time-lapse movie of microbial community consisting of wild type *E. coli* and the *E. coli* auxotroph ΔilvE growing in 1.0- μm deep, 0.3-0.6- μm wide, and 50- μm long tracks in 4.5% w/v agarose with minimal defined medium and glucose at 35C. The frame rate is 1 per 5 minutes, and the movie contains 6 hours of a 24 hour measurement.