Supporting Information

Deguchi et al. 10.1073/pnas.1018027108

SI Materials and Methods

Microbial Culture Under Hyperaccelerations. *P. denitrificans* ATCC17741^T (American Type Culture Collection) was incubated in LB broth containing 25 mM KNO_3 at 30 °C for 18 h, diluted 100-fold with LB broth without KNO_3, and incubated further until the A_{600} of the culture reached ∼0.7 (for approximately 4.5 h). The culture was diluted 100-fold with LB broth with KNO_3 to ∼10^6 cells/mL and incubated at 30 °C in a centrifuge. Control experiments at 1 × *g* and 30 °C were performed using the same medium in stationary culture.

E. coli W3110 was preincubated in LB broth at 37 °C for 15 h with shaking, diluted 100-fold with fresh LB broth, and incubated further with shaking until the A_{600} of the culture reached ∼0.7 (for approximately 1.8 h). The culture was diluted 100-fold with fresh LB broth to ∼10^6 cells/mL and incubated at 37 °C in a centrifuge. Control experiments at 1 × *g* and 37 °C were performed using the same medium in stationary culture.

S. amazonensis ATCC 700329^T (American Type Culture Collection) was preincubated in LB broth at 37 °C for 15 h with shaking, diluted 100-fold with fresh LB broth, and incubated further with shaking until the A_{600} reached ∼0.7 (for approximately 1.5 h). The culture was diluted 100-fold with fresh LB broth to ∼10^6 cells/mL and incubated at 37 °C in a centrifuge. Control experiments at 1 × *g* and 37 °C were performed using the same medium in stationary culture.

L. delbrueckii subsp. *delbrueckii* was preincubated in de Man, Rogosa, and Sharpe (MRS) (Difco) broth at 37 °C for 24 h without shaking, diluted 100-fold with fresh MRS broth, and incubated further without shaking until the A_{600} reached ∼0.7 (for approximately 9 h). The culture was diluted 100-fold with fresh MRS broth to ∼10^6 cells/mL and incubated at 37 °C in a centrifuge. Control experiments at 1 × *g* and 37 °C were performed using the same medium in stationary culture.

S. cerevisiae YPH499 was preincubated in yeast extract-peptone-dextrose (YPD) broth at 30 °C for 16 h with shaking, diluted 100-fold with fresh YPD broth, and incubated further with shaking until the A_{600} of the culture reached ∼0.7 (for approximately 9 h). The culture was diluted 100-fold with fresh YPD broth to ∼10^5 cells/mL and incubated at 30 °C in a centrifuge. Control experiments at 1 × *g* and 30 °C were performed using the same medium in stationary culture.

Cell Size Measurements. *P. denitrificans* was cultured at 30 °C and 134,425 × *g* for 48 h. After incubation, the culture was cooled in an ice bath and *P. denitrificans* cells were dispersed on a vortex mixer and harvested by centrifugation. The supernatant was discarded, and 2 mL of a fixing solution [2.5% (wt/vol) glutaraldehyde in PBS] was added to the pellet. The cells were then dispersed and fixed at 4 °C overnight. The fixed cells were redispersed, and 10 μL of the dispersion was deposited on a transmission electron microscopy grid. After drying in air overnight, the specimen was negatively stained with phosphotungstic acid and examined on a JEOL JEM-1210 (JEOL, Ltd.) operating at an incident beam energy of 120 kV. Cell dimensions were measured using "analySIS" (Soft Imaging System, GmbH). For measurements, a rectangular region-of-interest (ROI) was first generated. The ROI was positioned, size-adjusted, and rotated so that it enclosed a cell to be measured. The length and width of the cell were obtained as the length and width of the ROI. Measurements were performed on 1,460 and 1,155 cells for 1 and 134,425 × *g*, respectively.

Calculation of $P_{centrifuge}$. Acceleration of a sample in a centrifuge is described as

$$a_{rot} = r\omega^2 \quad [S1]$$

where r is the radial distance from the axis of rotation and ω is the angular velocity. In the case of fixed-angle rotors (Fig. S3), an average radial distance, r_{avg}, can be defined as

$$r_{avg} = \frac{1}{2} \times (r_{\max} + r_{\min}). \quad [S2]$$

The hydrostatic pressure to which microbial cells are subjected during centrifugation ($P_{centrifuge}$) is described as

$$P_{centrifuge} = P_{atmosphere} + \rho_{medium} \times r_{avg}\omega^2 \times (r_{\max} - r_{\min}) \quad [S3]$$

where $P_{atmosphere}$ is the atmospheric pressure and ρ_{medium} is the density of culture medium. Assuming that $P_{atmosphere}$ = 0.1 MPa and ρ_{medium} = 1 g·cm^{-3}, we estimated $P_{centrifuge}$ for different accelerations. Other parameters used in the calculations are given in Table S1.

Growth of *P. denitrificans* Under Elevated Hydrostatic Pressure. *P. denitrificans* was preincubated in LB broth at 30 °C with shaking (135 rpm) for 19 h. The cultures were inoculated in 3 mL of fresh LB broth, and the cells were grown to midexponential growth phase (A_{600} of 0.7) with shaking. The exponentially growing cultures were diluted with LB broth supplemented with 25 mM KNO_3 to ∼4 × 10^6 cells/mL The cultures were put into a series of sterilized polypropylene tubes, and the tubes were sealed with Parafilm (Pechiney Plastic Packaging). Each tube was placed in a stainless-steel hydrostatic chamber, and the appropriate hydrostatic pressure was applied using a hand pump. The chambers containing the culture tubes were incubated at 0.1, 30, and 40 MPa of hydrostatic pressure at 30 °C in a water bath. Each chamber was depressurized at certain intervals, and small aliquots of the cultures were taken out. The aliquots were diluted with physiological saline and inoculated on LB-agar plates. The plates were incubated at 30 °C for at least 50 h, and the number of colonies formed on the plates was enumerated. The rest of the cultures were repressurized and incubated further.

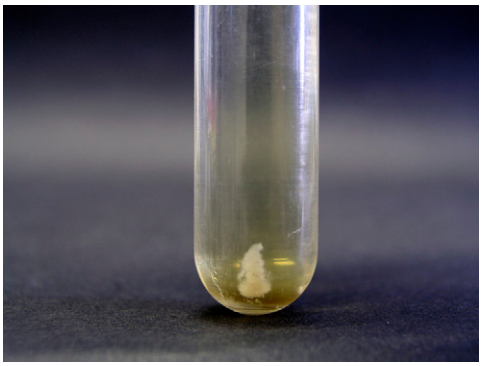

Fig. S1. Photograph of pellet of *E. coli* cells formed after incubation at 134,425 × *g* and 37 °C for 48 h. The outer diameter of the tube is 18 mm.

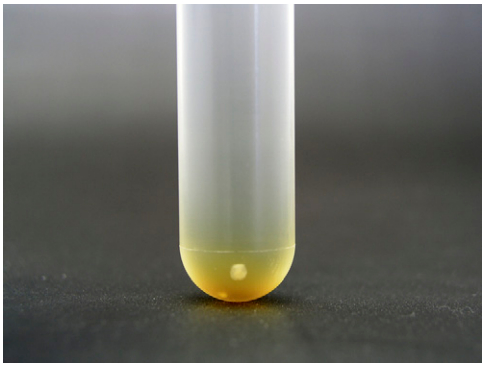

Fig. S2. Photograph of pellet of *E. coli* cells formed after incubation at 403,367 × *g* and 37 °C for 60 h. The outer diameter of the tube is 18 mm.

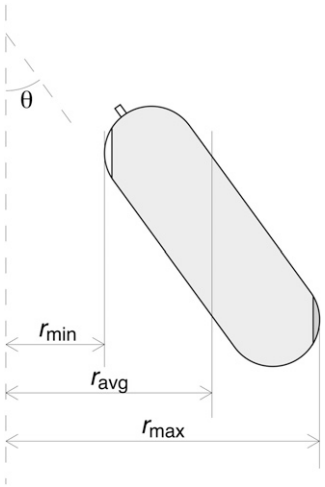

Fig. S3. Geometry of the centrifuge. A light gray shade represents a culture medium, and a dark gray shade represents a pellet of microbial cells.

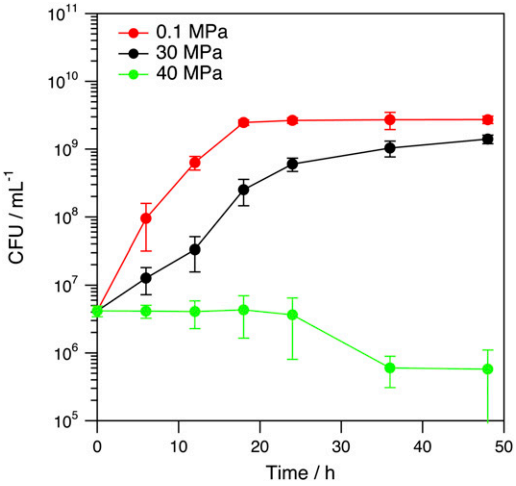

Fig. S4. Growth curves of *P. denitrificans* at 0.1, 30, and 40 MPa and at 30 °C as measured by colony counting on LB agar.

Table S1. List of parameters used for calculating hydrostatic pressures ($P_{centrifuge}$) under hyperaccelerations

g	r_{max} / mm	r_{min} / mm	r_{avg} / mm	ω / rpm
100 × g	106	34	70	920
1,000 × g	106	34	70	2900
7,500 × g	106	34	70	7,960
22,505 × g	104	35	69.5	13,900
29,819 × g	104	35	69.5	16,000
44,893 × g	104	35	69.5	19,632
52,375 × g	104	35	69.5	21,205
74,558 × g	104	35	69.5	25,300
134,425 × g	84	41	62.5	37,800
403,627 × g	84	41	62.5	65,500