Microbial growth at hyperaccelerations up to 403,627 × g

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It is well known that prokaryotic life can withstand extremes of temperature, pH, pressure, and radiation. Little is known about the proliferation of prokaryotic life under conditions of hyperacceleration attributable to extreme gravity, however. We found that living organisms can be surprisingly proliferative during hyperacceleration. In tests reported here, a variety of microorganisms, including Gram-negative *Escherichia coli*, *Paracoccus denitrificans*, and *Shewanella amazonensis*; Gram-positive *Lactobacillus delbrueckii*; and eukaryotic *Saccharomyces cerevisiae*, were cultured while being subjected to hyperaccelerative conditions. We observed and quantified robust cellular growth in these cultures across a wide range of hyperacceleration values. Most notably, the organisms *P. denitrificans* and *E. coli* were able to proliferate even at 403,627 × g. Analysis shows that the small size of prokaryotic cells is essential for their proliferation under conditions of hyperacceleration. Our results indicate that microorganisms cannot only survive during hyperacceleration but can display such robust proliferative behavior that the habitability of extraterrestrial environments must not be limited by gravity.

astrobiology | extremophiles

The robustness of prokaryotic life to physical extremes of temperature, pH, pressure, and radiation is well known (1) and has led to their ubiquitous presence on Earth (2, 3). Resilience to physical extremes is also extremely likely to be required for the existence of life beyond this planet (1, 4). Finding extraterrestrial life is a major motivation driving searches for extrasolar planets (5); thus, understanding the physical limits for known organisms is crucial in evaluating the probability that such planets harbor life (1, 4). Assessing the habitability of extraterrestrial environments requires an expanded set of criteria involving factors that can be ignored for terrestrial environments. The effect that extremes of gravity have on organisms is one such factor to consider when exploring for life beyond Earth.

The effect of microgravity on biological processes has been an active area of research particularly because it is relevant to human health during space flight (6, 7). Microorganisms make ideal model life forms for microgravity research because they are lightweight, small, and relatively easy to handle in space and have short generation times (7). Consequently, numerous experiments have been performed on microorganisms both in orbit and in Earth-based clinostats that simulate microgravity. The results demonstrate that microgravity affects microorganisms in a wide variety of ways related to their growth, physiology, pathogenesis, stress resistance, and gene expression (7–23).

The majority of these studies indicate that microgravity stimulates the growth of microorganisms (e.g., *Salmonella enterica* serovar Typhimurium, *Bacillus subtilis*, *Escherichia coli*) compared with 1 × g controls (8–17). In the case of *E. coli*, for example, the lag phase was shortened, the duration of exponential growth was increased, and the final cell population density was approximately doubled during space flights (13). Simulated microgravity can also affect the secondary metabolism of microorganisms. For example, production of β-lactam antibiotics by

Streptomyces clavuligerus, production of rapamycin by *Streptomyces hygroscopicus*, and production of microcin B17 by *E. coli* were suppressed during culturing in simulated microgravity, whereas production of gramicidin S by *Bacillus brevis* was unaffected (14, 18). *S. enterica* serovar Typhimurium showed enhanced virulence in a murine infection model (19, 20) conducted in space flight and under modeled microgravity compared with conditions of normal gravity (19, 20). These microorganisms also showed increased resistance to environmental stresses, increased survival in macrophages, and significant changes in protein expression levels (19). To elucidate the molecular mechanisms of microbial responses to microgravity, 2D gel electrophoresis and DNA microarray analysis have been used (19–23). Recent analysis of *S. enterica* serovar Typhimurium grown in space identified 167 transcripts and 73 proteins that changed expression compared with ground controls, and conserved RNA-binding protein Hfq was identified as a likely global regulator (20). Gene expression of eukaryotic *Saccharomyces cerevisiae* is also affected by simulated microgravity (22, 23).

Compared with the relatively active research on microbial responses to microgravity, there are fewer studies that report experiments on microorganisms exposed to gravities greater than 1 × g (11, 16, 24–28). Unlike in microgravity, experiments in hypergravity were performed exclusively in simulated environments and primarily by subjecting microorganisms to centrifugal acceleration in centrifuges. Bouloc and D'Ari (11) reported that hyperaccelerations of 3 and 5 × g did not affect the growth of *E. coli*, whereas Brown et al. (16) observed growth suppression at 50 × g. Similar observations were reported for *Paramecium tetraurelia*, which showed no effect at 10 × g but a significantly lower proliferation rate and a lower population density at 20 × g (24).

At hyperaccelerations much greater than ∼10^2 × g, the effect of sedimentation on microbial cells becomes significant. In a typical example, cultures of bacterial cells subjected to centrifugation at 3,000–5,000 × g for 5–10 min yielded pellets of intact bacterial cells (29). If microbial growth had occurred under these (or similar) conditions, it must have happened within or on the pellet. In stark contrast, the effect of cellular sedimentation is not very significant at lower accelerations, where growth can occur planktonically. Studying microbial proliferation, and not simply survival, at such hyperaccelerations addresses the fundamental biological question of what are the physical limits of organismic viability (1) under a range of gravitational accelerations larger than those found on Earth. Understanding the gravity limits for microorganism growth

has important implications in considering the emergence, transport, adaptation, and evolution of life in extraterrestrial habitats (4, 30).

Previous studies that dealt with microorganisms under accelerations much greater than $\sim 10^2 \times g$ focused mostly on survival, however. Spores of *B. subtilis* tolerate accelerations exceeding 10,000–15,000 $\times g$ for indefinite periods of time but were inactivated to a 10% survival rate when they were subjected for 65 h to 436,000 $\times g$ (25, 26). Inactivation of various microorganisms, including prokaryotic *E. coli*, *Thiobacillus intermedius*, *Bacillus amyloliquefaciens*, and *Staphylococcus aureus* as well as eukaryotic *S. cerevisiae*, was also studied after they were subjected to 450,000 $\times g$ (27). These studies were done in phosphate-buffered or physiological saline at 4 °C, wherein microbial proliferation was not possible even at $1 \times g$ because of the lack of nutrients and low temperature.

To our knowledge, the only study that has dealt with the proliferation of microorganisms under hyperaccelerative conditions was that of Montgomery et al. (28). In their experiments, *E. coli* suspended in nutrient broth at 35 °C was subjected to centrifugation at 1,000 or 110,000 $\times g$ for 24 h (28). They reported that the growth pattern of *E. coli* was not altered at 1,000 $\times g$. They found that growth was disturbed at 110,000 $\times g$, however. This was characterized by an increased duration of the lag phase, prolonged generation time, and decreased maximal cell concentration compared with $1 \times g$ controls. The study of proliferation of microorganisms under hyperaccelerations much greater than $\sim 100 \times g$ still remained largely unexplored, however (1). Here, we report that microorganisms can grow surprisingly well under hyperaccelerations and are able to proliferate even at 403,627 $\times g$.

Results

Growth of Microorganisms Under Hyperaccelerations. A variety of microorganisms were cultured in nutrient media under hyperaccelerations in centrifuges. The microorganisms studied here include Gram-negative *Paracoccus denitrificans, E. coli,* and *Shewanella amazonensis*; Gram-positive *Lactobacillus delbrueckii* subsp. *delbrueckii*; and eukaryotic *S. cerevisiae*. Fig. 1 *A–D* shows photographs of cultures of *P. denitrificans* in LB broth containing 25 mM KNO$_3$ after spinning in an ultracentrifuge at 403,627 $\times g$ and at 30 °C. At this acceleration, *P. denitrificans* cells sedimented and formed a pellet at the bottom of a centrifuge tube soon after centrifugation began. Initially, a pellet was not visible (Fig. 1*A*) because the total number of *P. denitrificans* cells in the culture was small ($\sim 10^6$ cells). A pellet of a visible size formed after spinning the culture for 6 h (Fig. 1*B*), however, and it increased in size with
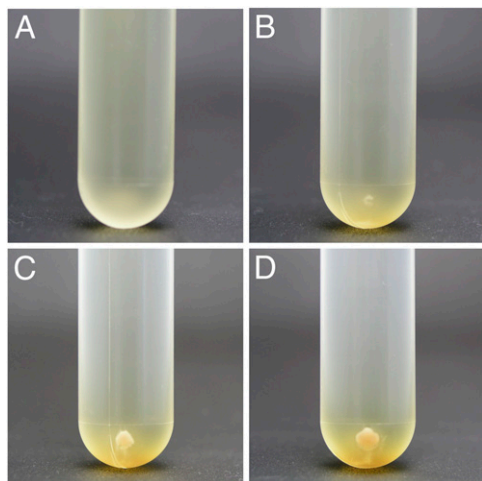
time (Fig. 1 *C* and *D*). The observation demonstrates unambiguously that *P. denitrificans* can proliferate even at 403,627 $\times g$.

Growth Characteristics of Microorganisms at Hyperaccelerations. To quantify the effect of hyperacceleration on growth characteristics of various microorganisms, growth curves were followed (Fig. 2). Pellets of microorganisms formed after incubation at hyperaccelerations were redispersed on a vortex mixer, and the number of live cells was counted by the spread plate technique.

The growth curves of *P. denitrificans* at 1 and 7,500 $\times g$ were identical within experimental error, indicating that hyperacceleration up to 7,500 $\times g$ did not affect the growth of *P. denitrificans* at all. The growth was slightly retarded when the incubation acceleration was increased to 74,558 and 134,425 $\times g$, as judged by the slight decreases in the respective final cell concentrations. At 403,627 $\times g$, a distinct lag phase appeared and the growth was retarded significantly. The final cell concentration was significantly smaller at this acceleration compared with those at lower values. The results again confirm the growth of *P. denitrificans* under a range of hyperaccelerations, although growth is retarded above 74,558 $\times g$.

Growth of *E. coli* under hyperaccelerations showed similar trends to that of *P. denitrificans*. The growth curve at 7,500 $\times g$ was identical to that at $1 \times g$. This observation is consistent with a previous study in which the authors reported no difference in the growth of *E. coli* at either 1 or 1,000 $\times g$ (28). We observed that growth was retarded at 22,505 $\times g$ but measured a final cell concentration identical to that at $1 \times g$. At 74,558 $\times g$, the growth was further retarded and the final cell concentration was lower. Formation of a macroscopic pellet was observed after incubation at 134,425 $\times g$ for 48 h (Fig. S1), again confirming growth and also consistent with the previously reported growth of *E. coli* at 110,000 $\times g$ (28). The cells were packed so tightly in the pellet that we were not able to redisperse them and determine the growth curve, however, whereas no such observation was described in the previous work (28). At 403,627 $\times g$, *E. coli* formed a pellet of barely visible size even after incubation for 60 h (Fig. S2), suggesting that growth at this acceleration is highly suppressed. The cells in the pellet could not be redispersed either.

An additional Gram-negative microorganism, *S. amazonensis*, showed identical growth curves when cultured at 1 and 7,500 $\times g$. At 22,505 $\times g$, however, a lag phase appeared, growth was retarded, and final cell concentrations became lower relative to those at $1 \times g$. These effects on growth became more significant as the incubation acceleration was increased beyond 22,505 $\times g$.

We found that growth of Gram-positive *L. delbrueckii* subsp. *delbrueckii* was very sensitive to hyperacceleration. The final cell concentration became noticeably lower even at $100 \times g$, and the growth rate became slightly smaller. Growth at 7,500 $\times g$ was identical to that at $100 \times g$. Very significant growth retardation was observed at 22,505 and 44,893 $\times g$, but we did not observe any distinct lag phase.

Growth of *S. cerevisiae*, the only eukaryote studied here, was also rather sensitive to hyperaccelerations, with evidence of growth retardation observed at 1,000 $\times g$. For *S. cerevisiae*, the retardation effect on growth gradually magnified up to 22,505 $\times g$. Very significant growth retardation was observed at 44,893 and 52,375 $\times g$, including significantly lower final cell concentrations compared with $1 \times g$. A visible pellet did not form at 74,558 $\times g$, indicating that growth was completely suppressed at this acceleration.

Generation Time of Microorganisms at Hyperaccelerations. To analyze the growth characteristics of different microorganisms under hyperaccelerations further, we obtained generation times from the observed growth curves. To make the comparison between different microorganisms easier, we calculated relative generation time (g_{rel}), which is defined as

$$g_{rel} = \frac{g_{hyperacceleration}}{g_{1g}} \qquad [1]$$

Fig. 1. Growth of *P. denitrificans* at 403,627 $\times g$. Photographs of pellet of *P. denitrificans* cells after incubation at 403,627 $\times g$ and 30 °C for 0 h (*A*), 6 h (*B*), 24 h (*C*), and 48 h (*D*). The outer diameter of the tube is 18 mm.
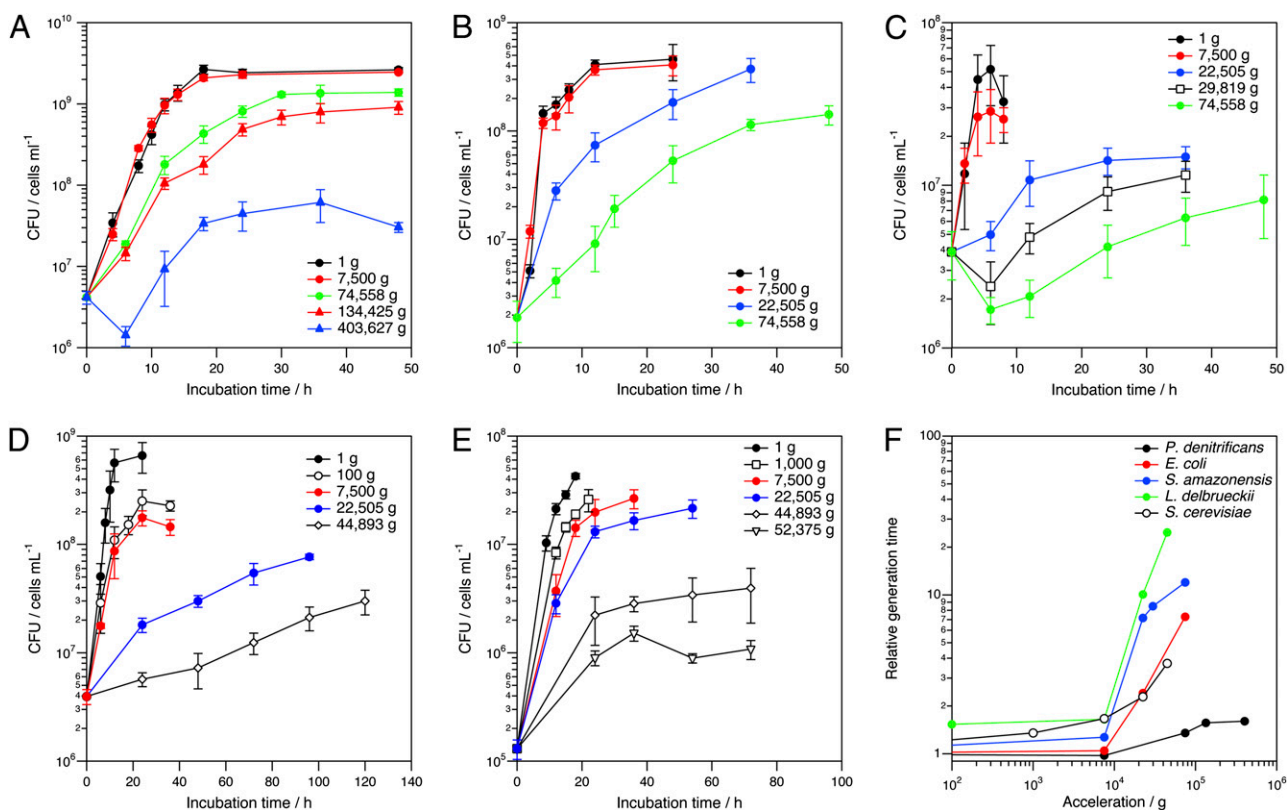
Fig. 2. Growth of various microorganisms under hyperaccelerations. (*A*) Growth curves of *P. denitrificans* at 30 °C and hyperaccelerations up to 403,627 × *g*. (*B*) Growth of *E. coli* in LB broth at 37 °C and hyperaccelerations up to 74,558 × *g*. (*C*) Growth of *S. amazonensis* in LB broth at 37 °C and hyperaccelerations up to 74,558 × *g*. (*D*) Growth of *L. delbrueckii* subsp. *delbrueckii* in MRS broth at 37 °C and hyperaccelerations up to 30,000 × *g*. (*E*) Growth of *S. cerevisiae* in yeast extract-peptone-dextrose broth at 30 °C and hyperaccelerations up to 74,558 × *g*. (*F*) Change in g_{rel} of various microorganisms as a function of acceleration.

where $g_{hyperacceleration}$ and g_{1g} are generation times of a given organism under hyperaccelerations and at $1 \times g$, respectively. The results are summarized in Fig. 2*F*.

For all prokaryotes tested here (i.e., both Gram-negative and Gram-positive bacteria), hyperacceleration had either no effect or a relatively small effect on growth below 7,500 × *g*, whereas growth was significantly retarded above ~$2 \times 10^4 \times g$. Among all prokaryotes tested here, the effect of hyperacceleration on growth was lowest for *P. denitrificans*. For the eukaryotic organism *S. cerevisiae*, the growth behavior observed here stands in contrast to that of prokaryotes in that the generation time became progressively longer with increased acceleration, particularly above 44,893 × *g*.

Possible Effect of Hyperacceleration on Microbial Growth. To understand how hyperacceleration affects microbial growth, we considered three aspects that differentiate the growth of microorganisms under hyperacceleration from that at $1 \times g$. These are sedimentation, mechanical deformation, and hydrostatic pressure.

Effect of Sedimentation. Under conditions of hyperacceleration, microbial cells grew in sedimented congested pellets. The density of cells within these pellets increased as gravity increased, leading to relatively smaller void volumes between cells in pellets formed at relatively high acceleration. Therefore, diffusion rates of small molecules in these pellets became progressively slower because of an increasing obstruction effect. Consequently, nutrient uptake and waste disposal by cells in the growing pellets were inhibited, thereby affecting the growth rates.

Conversely, differential sedimentation of subcellular moieties and the formation of other molecular concentration gradients within cells may also negatively affect growth under hyperaccelerative conditions. By analogy, centrifugation at $10^5 \times g$ is routinely used for sedimentation, and thus separation of relatively large particulates like ribosomes from the molecular milieu of cell lysates (31). In this study, a distinct concentration gradient of nutrient components in LB broth was observed when the culture of *P. denitrificans* was spun at 403,627 × *g* for longer than 6 h (Fig. 1 *B*–*D*). Yellow-colored components formed a sediment at the bottom of the centrifuge tube, leaving a colorless supernatant. A similar concentration gradient of cytoplasmic components may form within microbial cells at hyperaccelerations.

The concentration gradient of a particle subject to gravitational force at a sedimentation equilibrium is described by (32)

$$c_{(z)} \propto e^{-(m - V\rho_m)gz/k_B T} \qquad [2]$$

where m and V are the mass and the volume of the particle, ρ_m is the density of the medium, k_B is the Boltzmann constant, T is the absolute temperature, and z is the particle position relative to the bottom. We calculated concentration gradients of model proteins of various molecular masses (1 kDa, 10 kDa, 100 kDa, and 1 MDa) at 500,000 × *g* and 30 °C over a distance of 10 μm (Fig. 3*A*) within a model cytosol. Volumes of the respective proteins were calculated using an average value of the partial specific volumes of most proteins (0.73 cm³·g⁻¹) (33). A value of 1.1 g·cm⁻³ was used as an average representative density of the model cytosol (34).

If we consider sedimentation over 1 μm, which is a typical size for prokaryotic cells, the calculation shows that sedimentation is negligible for relatively small proteins having molecular masses of 1, 10, and 100 kDa. Significantly, even the 100-kDa protein had a concentration at the top of the cell ($z = 1$ μm) that was 96% of the value at the bottom ($z = 0$ μm). In contrast, a very profound effect of sedimentation was calculated for the 1-MDa protein, where the concentration at the top was 69% of that at the bottom. These analyses indicate that intracellular sedimen-

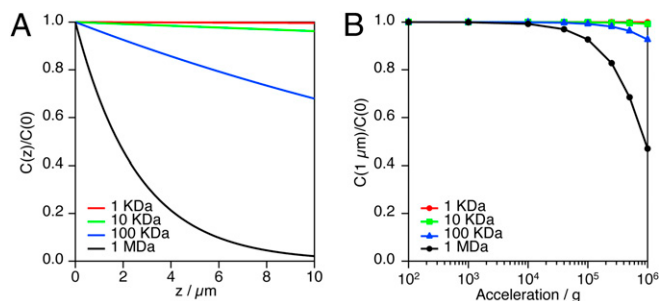MICROBIOLOGY

Fig. 3. Concentration gradient of model proteins within a cell. (*A*) Calculated concentration gradients of model proteins of different molecular masses (1 kDa, 10 kDa, 100 kDa, and 1 MDa) at the sedimentation equilibrium at 500,000 × *g* and 30 °C over a distance of 10 μm. (*B*) Sedimentation of model proteins of different molecular masses over a distance of 1 μm as a function of acceleration.

tation of large molecular complexes [e.g., bacterial 70S ribosome, a large nucleoprotein complex with a molecular mass of ~2.5 MDa (35)] may take place at 500,000 × *g*, thereby affecting relevant cellular processes.

Very different results were obtained when we consider sedimentation over a distance of 10 μm, which is a characteristic size for eukaryotic cells. In this case, a profound sedimentation effect was calculated even for 100-kDa proteins. Thus, intracellular sedimentation effects may be more significant in the nearly spherical cells of *S. cerevisiae* (*ca.* 8 μm in diameter) (36) compared with smaller prokaryotes. These results strongly indicate that the size of cells must be small, like that of prokaryotes, to be resistant to the formation of intracellular concentration gradients and molecular sedimentation under hyperaccelerations.

Fig. 3*B* shows the ratio of the concentration of hypothetical proteins at $z = 1$ μm to that at $z = 0$ as a function of acceleration. Hyperaccelerations up to $10^6 \times g$ appear not to have induced a sedimentation effect on model proteins with masses of 1 and 10 kDa. The sedimentation became noticeable at ~$10^5 \times g$ for a 100-kDa protein and at ~$10^4 \times g$ for a 1-MDa protein. It is interesting to point out that the very significant retardation of growth by hyperacceleration was also observed above ~$10^4 \times g$ for all the prokaryotic microorganisms studied (Fig. 2*F*). The similarity between these two results suggests that sedimentation of large molecular complexes, such as ribosomes, may be one reason for growth retardation under hyperaccelerations above ~$10^4 \times g$.

The generation time of eukaryotic *S. cerevisiae* showed different acceleration dependence compared with the prokaryotes (Fig. 2*F*). The difference could be attributed, in part, to the larger cell size of *S. cerevisiae*, which affects the sedimentation of proteins (Fig. 3*A*). In eukaryotic cells, however, sedimentation of their organelles is highly likely because these are significantly larger than either proteins or ribosomal complexes. Nuclei and mitochondria of *S. cerevisiae*, for example, can be pelleted easily by centrifugation at 600 × *g* for 5 min and at 10,000 × *g* for 2 min, respectively (37). Thus, during growth of *S. cerevisiae* at hyperaccelerations above 10,000 × *g*, these organelles could not have been distributed homogeneously in the cytoplasm as they are at 1 × *g*. Rather, they should have separated and clustered at the bottom of the cell. Although the effect was not lethal enough to suppress the growth completely, organellar sedimentation and clustering should have affected and retarded the cell growth.

Mechanical Deformation of Microbial Cells Under Hyperaccelerations. Macroorganisms easily collapse under accelerations of only a few times that of Earth's surface gravity (25). In contrast, microorganisms are likely to show higher resistance to mechanical deformation under hyperaccelerations because the gravitational potential is proportional to the size of an object. Indeed, Yoshida et al. (27) reported no change in the cell shape for *E. coli* and *B. amyloliquefaciens* after ultracentrifugation in physiological saline at 450,000 × *g* and 4 °C for 24 h. The effect of mechanical deformation on cells that replicate via binary fission at hyperacceleration was not known, however.

To evaluate the effect of hyperacceleration on cell morphology, *P. denitrificans* cells incubated at 30 °C for 48 h at both 1 and 134,425 × *g* were imaged by transmission electron microscopy. Distributions of cell length, width, and aspect ratio were then measured using image analysis software (Fig. 4).

Comparison of the images (Fig. 4 *A* and *B*) and histograms (Fig. 4 *D–I*) shows no significant differences in the morphology of both sets of cells. Occasionally, highly elongated cells were observed when incubated at 134,425 × *g* (indicated by a white arrowhead in Fig. 4*C*), but the population of such cells was minor, comprising less than 1% of the cells examined. These results suggest that prokaryotic cells are small enough (~1 μm) that irreversible mechanical deformation attributable to gravity is not significant.

Effect of Hydrostatic Pressure on Growth of Microbial Cells Under Hyperaccelerations. Microbial cells in the pellet at the bottom of the centrifuge tube were subjected to a hydrostatic pressure, $P_{centrifuge}$, because of the weight of the water column above the
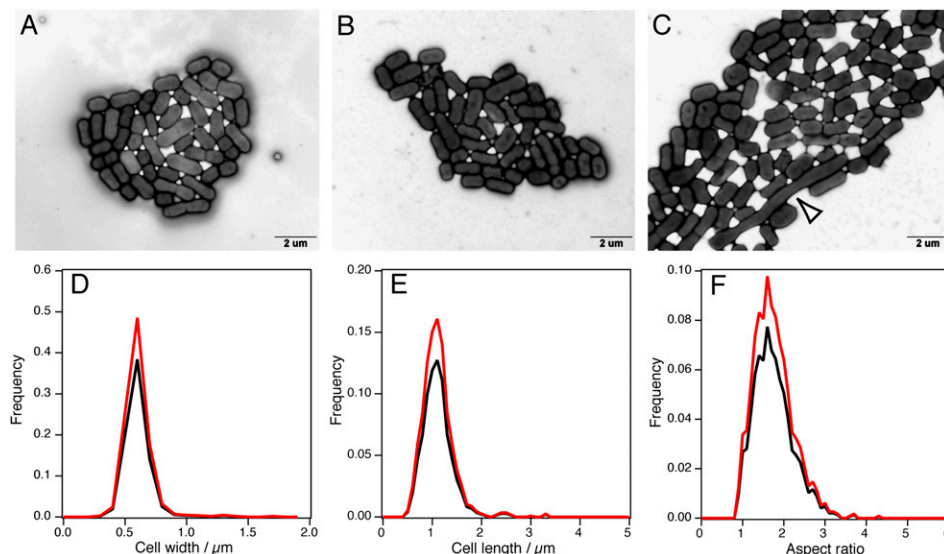

Fig. 4. *P. denitrificans* cells after incubation at hyperaccelerations. Transmission electron microscopy images of *P. denitrificans* cells after incubation at 30 °C and at 1 × *g* for 4 h (*A*) and 134,425 × *g* for 48 h (*B* and *C*). (*C*) Occasionally, highly elongated cells were observed when incubated at 134,425 × *g* (white arrowhead). (Scale bar: 2 μm.) (*D–F*) Size distribution of *P. denitrificans* cells after incubation at 1 × *g* (black) and 134,425 × *g* (red).

pellet. $P_{centrifuge}$ is negligibly small at $1 \times g$ but increases linearly with acceleration and becomes significant at hyperaccelerations. $P_{centrifuge}$ is estimated to be 0.1 MPa at $100 \times g$, 0.6 MPa at $1,000 \times g$, 3.6 MPa at $7,500 \times g$, 10.3 MPa at $22,505 \times g$, 13.6 MPa at $29,819 \times g$, 20.4 MPa at $44,893 \times g$, 23.7 MPa at $52,375 \times g$, 33.8 MPa at $74,558 \times g$, 42.2 MPa at $134,425 \times g$, and 126.5 MPa at $403,627 \times g$ (*SI Materials and Methods*, Fig. S3, Table S1).

For most mesophilic microorganisms, cell division is not affected at hydrostatic pressures below ~20 MPa (38). For example, the colony-forming ability of *E. coli* is suppressed only when it is cultured under hydrostatic pressures above 40 MPa (39). Thus, it is unlikely that hydrostatic pressure at hyperaccelerations up to $52,375 \times g$ plays a dominant role in the growth retardation under hyperaccelerations above ~$10^4 \times g$ (Fig. 2F).

Hydrostatic pressure may have had an impact on the growth of *P. denitrificans* above $74,558 \times g$, where hydrostatic pressure is estimated to be higher than 33.8 MPa. Very interestingly, we found that the growth of *P. denitrificans* was inhibited completely above 40 MPa (Fig. S4), suggesting that the observed growth here should not have occurred above $134,425 \times g$. Although the reason for this discrepancy is not clear at present, one possibility is attributable to the compositional gradient in the medium induced during sedimentation. As can be seen in Fig. 1 *B–D*, high-molecular-weight protein molecules may be advantageously accumulated at the bottom of the centrifuge tube along with *P. denitrificans*, providing local nutrients for growth. The discrepancy may also be ascribed to the difference in cell density during growth at elevated pressures compared with hyperaccelerations. *P. denitrificans* grew planktonically when it was cultured at elevated pressures, whereas the growth occurred in a densely packed pellet at hyperaccelerations. The pressure effect on cellular processes depends on cell density in some cases (40). Human dermal fibroblasts undergo a significant morphological change and become rounded when they are subjected to 70 MPa at a low cell density (subconfluence). No such change is observed when they are pressurized at a high cell density (full confluence), however. Higher cell density at hyperacceleration may therefore offset the pressure effect to some extent.

Discussion

Our results clearly demonstrate that microorganisms not only survive at hyperacceleration but can grow by binary fission, producing viable cells. All microorganisms studied here displayed growth in culture at hyperaccelerations up to ~$2 \times 10^4 \times g$. Because of the instrumental limitation of our ultracentrifuge, we did not determine the upper limit of acceleration for the growth of the most tolerant organisms. We did observe the proliferation of *P. denitrificans* and *E. coli* even at $403,627 \times g$, however. We argue that this latter finding is significant when trying to understand the limits of hypergravity tolerance for our version of carbon-based life. Because the list of bacteria and yeast here is a short one, we anticipate that further accumulation of experimental data on various microorganisms will result in discoveries of previously undescribed species that expand the range of habitable accelerations.

In an attempt to understand our various observations of microbial growth under hyperaccelerative conditions, we considered three most obvious effects, namely, sedimentation of cells, mechanical deformation of membranes, and hydrostatic pressure. Most notably, our analysis did not explain why some species are more tolerant. For example, we do not explain why only *P. denitrificans* and *E. coli* showed growth at $403,627 \times g$, whereas other species did not. This likely implies that species-specific biochemical processes led to the differential sensitivities that were observed. Our experiments were aimed at gaining an improved understanding of the physical effects of hyperaccelerative conditions on microbial cells rather than at elucidating subtle details of the biological processes likely affected by gravity, however (6, 41, 42).

Nonetheless, we briefly consider some biological processes in the hypergravity environment. Previous studies reported that the

physical effects of microgravity can be sensed by cell surfaces and transformed into biochemical responses (7). Cytoskeletal proteins and their polymerized superstructures, for example, may play important roles in gravity sensing of mammalian cells (43), and possibly of microbial cells (7). It has also been suggested that mechanical changes of cell membranes in microgravity conditions are sensed by mechanosensitive channels (7). Under conditions of hyperacceleration, congestive packing and jamming of cells within microbial pellets would likely induce critical changes in the curvature and/or internal bilayer stress within the microbial cell membranes. Consequently, changes in the conformation of various sensor proteins could then lead to direct cellular and quorum responses (of various kinds) to the hypergravity field.

Our findings compliment previous studies that primarily focused on hypogravity and relatively mild hypergravity by extending the range of studies well into the hypergravity regime. Especially relevant in this regard are our findings on the hypergravity tolerance of *E. coli* and several other microorganisms. Here, the growth of *E. coli* was not affected at all by hyperaccelerations up to $7,500 \times g$ and we even observed growth at $403,627 \times g$, although it was significantly retarded. This range is three orders of magnitude wider than most of the previous studies (10–16).

From a practical perspective, it has been suggested that altering the accelerative environment could be used to manipulate bacterial fermentation processes (44) via production and localization of microbial metabolites known to be affected by microgravity (14, 18). It is likely that hyperaccelerative conditions may also be used to induce unique metabolite production in growing cultures. For example, the suppressed biosynthesis of antibiotics reported under microgravity conditions compared with $1 \times g$ controls (14, 18) may be enhanced at elevated gravities.

Exploring the physical limits of organismic viability is crucial in the search for life in extraterrestrial habitats, because the knowledge effectively helps in narrowing down possible targets to search (4). This present study expands the limits for life into the hypergravity regime, where this had not been seriously considered before (1). We propose that this has significant implications for astrobiology. For example, microorganisms subjected to hyperaccelerations on the order of $10^5 \times g$ have attracted scientific attention in terms of bacterial transport between planets (panspermia). The hypothesized process begins with an asteroidal impact on a donating planet followed by consequent ejection of bacteria-bearing rocks (30). Under impact conditions, ejected rocks typically experience maximum accelerations of $3 \times 10^5 \times g$ and rise times of 0.5 ms in the case of (ejection from) Mars (26). Bacteria have to survive extremes in both acceleration and rate of change of acceleration (25). Dormant spores of *B. subtilis* are inactivated when they are subjected to ~$10^5 \times g$ (26). The inactivation follows first-order kinetics and decreases exponentially with exposure time, however (26). Our results show that hyperacceleration of ~$10^5 \times g$ is within a habitable range for some microorganisms. This significantly enhances the evidence that bacteria can remain robustly viable after asteroidal impact-style ejection.

Most significantly, our finding also extends the possibility of life beyond planets to massive substellar objects like brown dwarfs, the coldest of which has an effective surface temperature of ~400 K (45), which is extremely close in value to the known upper temperature limit for life (395 K) (46). The relatively strong gravitational fields associated with brown dwarfs are one of several limiting factors in considering existence of life on brown dwarfs (47). Our results unambiguously show that the ~$10–10^2 \times g$ gravitational fields existent on relatively cold (~600 K) brown dwarfs (48) must not be a primary limiting factor in assessing their potential for harboring life as we know it.

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. *E. coli* W3110 and *S. amazonensis* ATCC 700329[T] (American Type Culture Collection) were incubated in LB broth at 37 °C. *L. delbrueckii* subsp. *delbrueckii* was incubated in de Man, Rogosa, and Sharpe

(MRS) broth (Difco) at 37 °C. *S. cerevisiae* YPH499 was incubated in yeast extract-peptone-dextrose broth at 30 °C. Detailed culture conditions are available in *SI Materials and Methods*.

Incubation of microorganisms under hyperaccelerative conditions was performed using either an EIX-135 centrifuge (TOMY Seiko) for incubation below $7,500 \times g$ or an XL-80 ultracentrifuge (Beckman Instruments) for incubation above $22,505 \times g$. The accelerations reported are the maximum values achieved at the bottom of the respective centrifuge tubes in which microbial growth occurred. Control experiments at $1 \times g$ were performed in stationary culture.

Determination of Growth Curves. Immediately after incubation, the centrifuge tubes were taken out and cooled in an ice bath. The microbial cells were dispersed on a vortex mixer. The culture was diluted with physiological saline to $\sim10^5$ cells/mL and spread on agar plates containing the same culture media used for culture under hyperaccelerative conditions, with the exception of *P. denitrificans*, for which KNO_3-free LB broth was used. The number of colonies formed was enumerated after incubation at $1 \times g$ and at the same temperature used for incubation at hyperaccelerations.

Cell Size Measurements. *P. denitrificans* was cultured at 30 °C and $134,425 \times g$ for 48 h. After incubation, *P. denitrificans* cells were fixed with glutaraldehyde, negatively stained with phosphotungstic acid, and examined on a JEOL JEM-1210 (JEOL, Ltd.). Cell dimensions were measured using "analySIS" (Soft Imaging System, GmbH). Measurements were performed on 1,460 and 1,155 cells for $1 \times g$ and $134,425 \times g$, respectively.

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