Transformation Frequency of *Escherichia coli* HB101 under Low-Shear Modeled Microgravity

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Abstract

Viable microorganisms are frequently found in space stations. The unique adaptive ability of bacteria partly depends on horizontal gene transfer among the individual cells. Microgravity, defined as a local environment in a space habitat where gravity seems not to act, is an unexperienced condition for most of Earth’s organisms, and can therefore affect bacterial physiology, gene expression, and gene transfer. The present study evaluates the effect of microgravity on horizontal gene transfer in *Escherichia coli*. To this end, we compared the transformation frequencies of *E. coli* under normal gravity and under low-shear modeled microgravity (LSMMG) generated by a high-aspect rotating vessel (HARV). Our results demonstrated that bacterial transformation is not hampered by LSMMG, and the potential risk of bacterial gene transfer during space flight is comparable to that on Earth. Therefore, we should arrest the spread of harmful genes such as toxin-producing and antibiotic resistance genes in crewed space habitats, as well as on the Earth. ©2015 Jpn. Soc. Biol. Sci. Space; doi: 10.2187/bss.29.19

Introduction

Previous studies have demonstrated the presence of viable microorganisms in space stations such as Sky Lab, Mir, and the International Space Station (ISS) (Castro et al., 2004; Novikova, 2004; Ott et al., 2004; Novikova et al., 2006; Van Houdt et al., 2012). Microorganisms within space habitats pose hazards to crew health and can potentially damage the flight hardware (Yamaguchi et al., 2014). The space habitat is a unique closed environment where gravity is distinctly low (microgravity), the intensity of cosmic radiation is high, and water and air are regenerated and reused. The ecosystem in the ISS completely differs from earthly ecosystems. Because the components of the present-day ISS ecosystem are limited to humans and microbes, microbes may affect humans more severely than in ground habitats. In addition, the pathogenicity and virulence of some bacteria, such as *Salmonella enterica* serovar Typhimurium, have been shown to increase during spaceflight (Wilson et al., 2007). The immune system responses of astronauts are often weakened during spaceflight (Borchers et al., 2002), possibly due to the stress associated with life in confined quarters. Therefore, the risk of infectious diseases, especially opportunistic infection, is thought to be increased in space habitats. The importance of rapid and robust environmental monitoring is clearly noted in the mission roadmaps of the international partners on the ISS (National Aeronautics and Space Administration [NASA], 2010; European Space Agency [ESA], 2012; Japan Aerospace Exploration Agency [JAXA], 2012). For continued success in long-duration space habitation, astronauts must be provided with essential information on microbiological safety (e.g., the correct upper and lower safety thresholds of microbes in the air, surface, and water) (Ott et al., 2014). In fact, NASA, the Russian Federal Space Agency, and JAXA are continually monitoring microbial presence in the ISS to determine the abundance and analyze the community structures of bacteria colonizing the station (Venkateswaran et al., 2014; Yamaguchi et al., 2014). However, our knowledge of bacterial responses to crewed environments in space is limited and remains unclear (Ott et al., 2014).

Microgravity, defined as a local environment in a space habitat where gravity seems not to act, is foreign to most of the organisms living on Earth. Microgravity can impact on bacterial physiology (Rosenzweig et al., 2010) and gene expression (Wilson et al., 2007), and bacteria must adapt to microgravity when left in a space habitat. Bacteria have a unique adaptability to various environmental conditions, which is crucial for survival in severe environments. One of the most outstanding characteristics of bacteria is horizontal gene transfer. Bacteria can inter-exchange tens to thousands of kilobases of DNA and thereby diversify their genomes (Andam et al., 2011; Skippington et al., 2011; Wiedenbeck and Cohan, 2011). Therefore, by investigating bacterial horizontal gene transfer under microgravity, we can show microbial response to unexperienced environments and evaluate the risk of harmful genes (such as toxin-producing and antibiotic resistance genes) spreading through space habitats.

In this study, we determined the transformation frequency of *Escherichia coli* under low-shear modeled microgravity (LSMMG) generated by a high-aspect rotating vessel (HARV) rotary cell culture system, which was developed by NASA (Prewett et al., 1993) to simulate microgravity (Nickerson et al., 2004; Herranz et al., 2013). To evaluate the effect of microgravity on horizontal gene transfer in *E. coli*, we compared the transformation frequencies under LSMMG and under normal gravity. We selected *E. coli* because this organism constitutes part of the human gut microbiota (human-associated bacteria), and has often been found in space stations.
Materials and methods

Preparation of E. coli culture for transformation

E. coli cells were prepared for transformation following a standard protocol. Super Optimal Broth (SOB) and Luria Broth (LB) media were prepared as described by Hanahan (1983). Transformation buffer (TB; 10 mM PIPES, 55 mM MnCl₂, 15 mM CaCl₂, 250 mM KCl [pH 6.7]) was prepared as described by Inoue et al. (1990). E. coli HB101 cells were inoculated in 200 mL SOB medium and cultured at 18 °C with vigorous shaking until the OD₆₀₀ increased to 0.6. This culture was then placed on ice for 10 min, transferred to a 200-ml centrifuge bottle, and spun at 2,500 G for 10 min at 4 °C. The pellet was resuspended in ice-cold TB and again spun down as described above. The cell pellet was gently resuspended in TB to a final concentration of 1.0 × 10⁸ colony forming units (cfu) ml⁻¹; then, dimethyl sulfoxide was added with gentle swirling to a final concentration of 7%. Finally, this suspension was placed on ice for 1 h.

Plasmid uptake during incubation in HARV

Plasmid DNA pGFPuv (Clontech, Mountain View, CA, USA), carrying the gfpuv and ampicillin resistance genes, was added to the prepared E. coli suspension at a final concentration of 1.5 × 10¹¹ copies ml⁻¹. The suspension was then transferred to vessels for HARV incubation. Each vessel was completely filled with the sample, and confirmed to be completely free of air bubbles. On the HARV system (Fig. 1A; Synthecon, Houston, TX, USA), the cells were positioned either vertically (for incubation under LSMMG conditions) or horizontally (for incubation under normal gravity) (Fig. 1B).

All samples set on the HARV system were incubated at 4 °C to inhibit bacterial cell division (leading to increased cell numbers) during incubation. The rotation rate was set to 25 rpm to facilitate the LSMMG condition. The vessels were fitted with a gas-permeable membrane, allowing constant air exchange during the incubation. At one-day intervals throughout the incubation, a few mL of sample was taken from the HARV cultures and inoculated in warm LB medium followed by incubation at 37 °C for 1 h. Total culturable E. coli cells and transformants were counted as described next.

Enumeration of E. coli cells and transformants

Total culturable E. coli cells and transformants were counted by spreading the cell suspension onto LB agar plates without ampicillin and onto LB agar plates containing 50 µg ml⁻¹ ampicillin, and incubating both plates for 24 h at 37 °C. Morphological information of the E. coli cells in each sample was obtained by fluorescence microscopy with 4′,6-diamidino-2-phenylindole (DAPI) staining (Yamaguchi and Nasu, 1997). For this purpose, approximately 10 µl of bacterial suspensions were collected from each of the vessels into microtubes, and DAPI (Nacalai Tesque, Kyoto, Japan) was added (final concentration: 1 µg ml⁻¹). After staining for 5 min with DAPI, the fluorescently-stained cells were trapped onto 0.2 µm-pore size polycarbonate membrane filters. The filters were mounted in immersion oil for observation by fluorescence microscopy (E-400; Nikon, Tokyo, Japan). More than 10,000 cells were observed under ultra-violet excitation to confirm the cell shape, and under blue excitation to detect the fluorescence of green fluorescent protein (GFP).

Statistical analysis

Student’s t test was used to determine significant differences between the densities of the incubated cells under LSMMG and under normal gravity. The significant differences between transformation frequencies under LSMMG and under normal gravity was also determined by Student’s t test.

Results and discussion

We first determined the suitable conditions (cell density, plasmid DNA concentration and HARV incubation temperature) for transformation under LSMMG. At E. coli cell densities below 10⁸ cfu ml⁻¹, no transformants were detected on the LB agar plates containing 50 µg...
ml⁻¹ ampicillin. Moreover, at plasmid DNA concentrations below 10⁻¹¹ copies ml⁻¹, the number of transformants was below the quantification limit throughout the HARV incubation. We therefore decided the E. coli cell density as 10⁸ cfu ml⁻¹ and the plasmid DNA concentration as more than 10⁻¹⁰ copies ml⁻¹. The number of culturable E. coli cells increased during HARV incubation at 25 °C but did not significantly change during incubation at 4 °C. To accurately determine the transformation frequency, bacterial cell division should be avoided during the incubation; for this reason, we incubated samples at 4 °C in the following experiments.

The transformation frequencies under LSMMG and normal gravity were determined by mixing E. coli HB101 cells with pGFPuv and incubating the mixtures on HARV. The densities of the colony-forming bacteria and colony-forming transformants throughout the incubation are plotted in Fig. 2. In this study, the quantification limit was 1.2 × 10³ cfu ml⁻¹. The densities of the incubated cells were slightly higher under LSMMG than under normal gravity after 1 day (P < 0.05 for all samples). E. coli cells lose their viability when incubated at 4 °C; therefore, the number of culturable E. coli decreased under normal gravity conditions. Conversely, the number of culturable E. coli cells remained more-or-less constant throughout the LSMMG incubation. Kim et al. (2014) reported that the optical density of LSMMG-cultured E. coli O157:H7 cells was 1.3 times higher under LSMMG than under normal gravity. Both cultures had been nurtured for 24 h in minimal media at 37 °C (the optimal temperature for E. coli growth). These data indicate that microgravity not only maintains the viability of E. coli cells, but may enhance their growth at suitable temperatures. Demain and Fang (2001) reported that bacteria such as E. coli and Bacillus brevis alter their metabolic properties during microgravity. Fluorescence microscopy confirmed that the shapes of the E. coli cells in each vessel were not significantly changed throughout the HARV incubation (data not shown).

The number of transformants exceeded the quantification limit during days 2–4 under LSMMG, but remained slightly below the quantification limit at day 4 under normal gravity (Fig. 2). However, the transformation frequencies were similar under LSMMG and normal gravity (Table 1; P > 0.05 for days 2 and 3). The transformation frequency was higher on day 3 than on day 2 under both gravity conditions. As the transformation of E. coli cells is not hampered by LSMMG, it can occur in confined space habitats at frequencies similar to those on Earth. To confirm the transfer frequency derived from the colony counts, we tried to count the GFP-expressing cells by fluorescence microscopy. However, although we could detect the GFP-expressing cells, very few of the cells showed green fluorescence (less than 5 among approximately 10,000 cells); thus, it was difficult to obtain quantitative results. The transformation frequencies decreased after 4 days, probably because the metabolism of E. coli altered during prolonged incubation at 4 °C.

Several studies based on HARV have clarified the effect of LSMMG on bacterial physiology and gene expression. We demonstrated that LSMMG does not reduce the transformation frequency of E. coli cells. Beuls et al. (2009) focused on conjugation, another route of horizontal gene transfer among bacterial cells, and determined the conjugation frequency of HARV-incubated Bacillus thuringiensis. They reported that under microgravity, conjugation occurs at a frequency similar to that under normal gravity. Together with the data in Beuls et al. (2009), our findings highlight the potential risk of

![Fig. 2. Number densities of E. coli HB101 and transformants incubated with pGFPuv in HARV under modeled microgravity and normal gravity. Error bars are the standard deviations (n = 4); µG and nG denote microgravity and normal gravity, respectively. * indicates densities below the quantification limit (1.2 × 10³ cfu ml⁻¹).](image_url)

### Table 1. Transformation frequencies of E. coli HB101 incubated under modeled microgravity and normal gravity (n = 4).

<table>
<thead>
<tr>
<th>Incubation period (day)</th>
<th>0 day</th>
<th>1 day</th>
<th>2 day</th>
<th>3 day</th>
<th>4 day</th>
<th>5 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modeled microgravity</td>
<td>–</td>
<td>–</td>
<td>0.78±0.36*</td>
<td>2.2±0.67</td>
<td>1.0±0.82</td>
<td>–</td>
</tr>
<tr>
<td>Normal gravity</td>
<td>–</td>
<td>–</td>
<td>0.75±0.11</td>
<td>2.5±0.60</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* Under quantification limit

* mean ± standard deviation

Unit: x10⁻¹
bacterial gene transfer during spaceflight; moreover, the level of such gene transfer is comparable to that on Earth. Lynch et al. (2006) reported that *E. coli* cells form thicker biofilms under microgravity than under normal gravity, and developed increased resistance to antibiotics such as penicillin and chloramphenicol. Therefore, we should prevent the spread of toxin-producing and antibiotic resistance genes in crewed space habitats as well as on the Earth.

In this study, we determined the gene transfer frequency in *E. coli* cells. Further experiments using bacteria isolated from the ISS will help us to clarify the effect of microgravity on transformation, and to evaluate the risk of gene transfer in space habitats.

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References


