Survival of soil microbial community exposed to hyper-gravity conditions

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Research article

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Abstract

Earth is the cradle of mankind, but it is impossible for human beings to live in the cradle forever. Sending soil microbial spores through space to foreign planets will be a likely initial process in planet colonization. Periods of hyper-gravity are likely to be a challenge for the candidate microorganisms during their interstellar transportation, raising questions about their survival rates and community-level responses. To address these questions, the impacts of hyper-gravity on soil microbial community composition and activity were tested by applying 1×g or 2500×g centrifugal force to soil for 6 days. The results indicated an increased diversity and absolute abundance of soil total bacterial community and a relatively stable active bacterial community under hyper-gravity condition. Besides, hyper-gravity had no observable effect on the relative abundance of soil microorganisms. These results suggest that soil microorganisms could survive during short periods of hyper-gravity. Our findings represent the first step towards a better understanding of the potential for survival of soil microbiomes during space travel and provide a basis for further interstellar soil research.

Introduction

The surge in population will not only cause food problems and lead to a food crisis in thirty countries around the world[1], but also result in the rapid consumption of energy and materials, destruction of the ecological environment, and even accelerated extinction of species. Humans have to look for the possibility of extraterrestrial life and assess the possibility of migrating from Earth to other celestial bodies[2]. There is no doubt that it is much easier to use microbes than humans during the search for life in space. We focus on searching for life in the solar system. About 60 years ago, six probes reached the surface of Venus one after another. Venus has a thick carbon dioxide atmosphere, the surface temperature is higher than the melting point of lead, indicating no possibility for life[3]. Mars is almost completely barren, very cold and dry. Microbes can survive in such surface conditions but it still not suitable for human habitation [4, 5]

Interstellar travel is a substantial challenge for these seeds, especially in a hyper-gravity environment. These seeds will have to go through three stages: ejecting from the planet of origin, passing through space and entering the atmosphere. After undergoing extreme acceleration, they will be exposed to extreme vacuum and ultraviolet. These processes are harmful to organisms[6]. The influence of extreme physical environments are key factors in the search for extraterrestrial life[7]. With the advancement of science and technology, microorganisms are getting more attention. Related studies have shown that microgravity can affect microorganisms in many aspects, including growth, gene expression and metabolic response[8–10]. Previous studies have reported that hyper-gravity can directly affect *Paramecium*, which is single-celled organisms, and inhibit its proliferation even for 1.8×g [11–13]. The growth of *Escherichia coli* was not affected at the gravity of 3×g and 5×g for 36 h[14]. Although the proliferation of *E. coli* was disturbed at 11,000×g when measured in 1963[15], this species has been shown to proliferate even at accelerations greater than 400,000×g[7]. It is believed that *Bacillus subtilis* can tolerate an acceleration of 436,000×g and last for 65 hours[16]. Some microbes, including

Paramecium tetraurelia and *E. coli*, can tolerate an acceleration of 450,000×g for 24 h[17]. However, there are still few studies on reported experiments of microorganisms exposed to gravity greater than 1×g, soil microorganisms in particular [18, 19].

It is well known that microbes are the most numerous, widely distributed, and capable of exploiting extreme environments[20]. Although ultraviolet radiation limits air-born microbes to some degree, soil microbes have substantial preservation opportunities[21]. Hyper-gravity conditions can be used to explore the physical limits of living body vitality, which is of great significance for considering the migration, adaptation and evolution of extraterrestrial environments. Ultracentrifuge can be used directly to successfully simulate different hyper-gravity conditions[22]. Previous studies have shown that rice seeds can survive in the space environment[23, 24], much less is known about the effect of hyper-gravity on the paddy soil microbial community. So, paddy soil was selected to research the changes in the composition of soil total and active microbial communities. Here, we report that soil total and active microorganisms can survive at hyper-gravity and also have a little rich community diversity Understanding the impact of gravity on soil microbial growth is important for considering the emergence, adaptation and evolution of life in extraterrestrial habitats[25].

Materials And Methods

Soil sampling and experimental design

The soil samples in this experiment were collected in Xiuzhou District, Jiaxing City, Zhejiang Province of China (30°50'8.74"N, 120°43'3.68"E), which was the most widely distributed paddy soil in the plain of Zhejiang Province. The basic physicochemical properties have been described previously[26]. Farmland is the representative area of rice cultivation in the Yangtze River Delta in China, which is cropped twice a year. In a 20 m × 20 m field, the "S" sampling strategy was adopted, and the surface soil with a depth of about 20 cm was collected with shovels. All soil samples were transported at 4 ℃ back to the laboratory, sieved with a 2 mm mesh and stored at -20 ℃ for further analysis.

To achieve the required gravity under laboratory condition, 1 g saturated paddy soil was subjected to ultracentrifugation (Thermo-Fisher Scientific, Shanghai, China) at different rotate speed. The experiment consisted of two treatments: (i) centrifugal for one hour under 2500×g first and then stood 6 days (2500g_DNA_0 or 2500g_RNA_0), (ii) 2500×g centrifugal force for 6 days (2500g_DNA or 2500g_RNA), and 1×g centrifugal force for 6 days as control. There are five analytical replicates per treatment. The ultracentrifuge was operating continuously. 2500g_DNA_0, 2500g_RNA_0 treatments and control samples were stored at the room temperature to keep identical environmental conditions. The centrifuge of 2500g_DNA and 2500g_RNA treatments was stopped at day 6 and stored with other group samples at -20°C for further analysis.

Soil DNA, RNA extraction and real-time PCR

DNA was extracted from treated soil samples with the MP FastDNA Spin Kit for Soil (MP Biomedicals, LLC, Ohio, USA) according to the manufacturer's instructions. Soil total RNA was extracted with E.Z.N.A.™ Soil RNA Mini Kit (Omega Bio-tek, Norcross, GA, USA) and purified with E.Z.N.A.™ RNase-Free DNase I Set (Omega Bio-tek, Norcross, GA, USA) according to manufacturer's protocols. After extraction, RNA was reverse transcripted to complementary DNA (cDNA) by PrimeScript™ II 1st strand cDNA Synthesis Kit (Takara, Dalian, China) for real-time PCR. The purity and concentration of DNA and RNA were determined using a NanoDrop 2000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA) and then stored at -20°C for microbial diversity and sequence analysis.

The real-time PCR assay was performed using the primer 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 907R (5'-CCGTCAATTCCTTTGAGTTT-3'). The 20 μL reaction systems contained: 1 μL of template DNA or cDNA, 10 μL of SYBR, 0.1 μL of each primer (100 μmol/L), and 8.8 μL of nuclease-free water. A standard curve was constructed using plasmids containing the 16S rRNA gene fragment. The PCR cycling started with an initial denaturation at 95°C for 3 min, followed by 27 cycles of 30 s at 95°C, 30 s at 55°C and 45 s at 72°C, and finally at 72°C for 10 min.

16S rRNA gene sequencing and statistical analysis

The profiling of the samples was conducted by sequencing the V4-V5 region of the 16S rRNA gene. Sequencing libraries were generated using NEBNext® Ultra™ DNA Library Prep Kit for Illumina® (New England Biolabs, MA, USA) according to the manufacturer's recommendations. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Fisher Scientific, MA, USA) and Agilent Bioanalyzer 2100 systems (Agilent Technologies, Waldbronn, Germany). The library was sequenced on an Illumina_Hiseq2500 platform and paired-end reads of length 250 bp were generated (Guangdong Magigene Biotechnology Co., Ltd. Guangzhou, China). Sequences of each sample were merged, trimmed, filtered, aligned, and then clustered into operational taxonomic units (OTUs) with VSEARCH-2.11.1[27] using a sequence similarity threshold of 0.97. Ribosomal database project (RDP) training set v16 [28] was used for taxonomy annotations at a threshold of 0.8 using the SINTAX algorithm[29]. The alpha-diversity indices were calculated by the function diversity () in the R package vegan[30]. Kruskal-Wallis tests were conducted to evaluate differences between treatments. Principal coordinates analysis (PCoA) using the Bray-Curtis distance to determine the differences in microbial communities between treatments. Permutational multivariate analysis of variance (PERMANOVA) was performed to measure the β-diversity significance using the function adonis() in vegan[30].

Results

Effects of different gravity conditions on alpha diversity of soil microbial community

To assess how total and active soil bacterial community were affected by different gravity treatments, we determined the α-diversity indices (the observed OTU richness, Shannon index, Chao1 and Phylogenetic

diversity). The Shannon indices of total soil bacterial community were significantly increased in hyper-gravity conditions (2500g_DNA_0 and 2500g_DNA) compared to control (1g_DNA) (Fig. 1A, $p < 0.05$). The Chao1 index was significantly higher in the treatment of 2500g_DNA_0 than that in 1g_DNA (Fig. 1B, $p < 0.05$). In contrast, the phylogenetic diversity and OTU richness of total soil bacterial community were not significantly different among hyper-gravity treatments (Fig. 1C, D). No significant effects of hyper-gravity conditions on active soil microbial community diversity were observed (Fig. 1). Under the same hyper-gravity conditions, there were significant differences between the diversity indices of total soil bacterial community and active ones, except for the OTU richness index between 2500g_DNA and 2500g_RNA, and the Shannon index between 2500g_DNA_0 and 2500g_RNA_0 (Fig. 1, $p < 0.05$).

Effects of different gravity conditions on beta diversity of soil microbial community

PCoA was performed to determine whether the gravity conditions affect the distribution of total and active soil microbial communities. The total and activate bacterial communities differed significantly under all gravity conditions using relative and absolute quantification methods, which were confirmed by the PERMANOVA of Adonis test ($R^2 = 0.46$, $p = 0.001$) and ($R^2 = 0.86$, $p = 0.001$), respectively (Fig. 2A, B). As for the relative quantification profile, the active microbial community was significantly separated by different gravity treatments but not in the case of total microorganisms (Fig. 2A, $R^2 = 0.21$, $p = 0.018$). In contrast, 2500g_DNA_0 was separated from 1g_DNA and 2500g_DNA along the principal coordinate 2 (PCoA2) in the absolute quantification profile (Fig. 2A; $R^2 = 0.96$; $p = 0.001$).

Effects of different gravity conditions on soil total microbial community composition

A total of 7,971 operational taxonomic units (OTUs) were detected for the total soil bacterial community, which was dominated by phyla of *Proteobacteria* (26.21%), *Chloroflexi* (23.37%), *Acidobacteria* (11.19%), *Thaumarchaeota* (4.90%) and *Verrucomicrobia* (3.95%) (Fig. 3A, B). The relative abundance of *Thaumarchaeota* significantly increased in the gravity of 2500g_DNA compared to that in 1g_DNA or 2500g_DNA_0 conditions (Fig. 3A, $p < 0.05$), while the other phyla showed no significantly different among the treatments. Regarding the absolute quantification profile, the abundance of bacterial communities was about $1.20 \times e^{12}$,$1.19 \times e^{12}$ and $2.70 \times e^{12}$ in 1g_DNA, 2500g_DNA_0 and 2500g_DNA, respectively (Fig. 3B). All the bacterial phyla displayed a significantly elevated abundance under the 2500 × g gravity compared to those in the other two treatments (Fig. 3B, $p < 0.05$).

Effects of different gravity conditions on soil active microbial community composition

The most abundant active soil bacterial taxa belonged to the phylum *Proteobacteria* (32.81%), *Chloroflexi* (11.55%), *Acidobacteria* (6.76%), *Bacteroidetes* (3.96%) and *Verrucomicrobia* (3.32%) (Fig. 4A). The relative abundance of *Bacteroidetes* was significantly higher under the 2500 × g gravity than that in 1g_RNA treatment (Fig. 4A, $p < 0.05$), but not for other phyla. The quantified bacteria abundance was about $1.54 \times e^{13}$,$1.42 \times e^{12}$ and $1.25 \times e^{13}$ in 1g_RNA, 2500g_RNA_0 and 2500g_RNA, respectively (Fig. 4B). The results showed that quantified abundances of active microorganisms were not significantly different among the treatments (Fig, 4B).

Discussion

Exploring the physical limits of the viability of soil microorganisms is crucial to find life in extraterrestrial habitats. Our findings extend the effect of hyper-gravity on a single microorganism to the microbial communities. Our results demonstrate that soil total and active microorganisms not only survive at hyper-gravity up to 2500× g but have a relatively rich community diversity. These were consistent with previous hyper-gravity studies indicated that *E. coli* was not affected by hyper-accelerations at 3, 5×g[31] and even at 7,500×g[8]. Similar results were observed for *P. tetraurelia*, which shows no influence at 10×g but a significantly lower population density and proliferation rate at 20×g[32]. The effect of microgravity on living organisms, especially microorganisms, has been an important research area as it provides a theoretical basis for searching life in extraterrestrial habitats[11, 33]. Numerous studies indicated that microgravity affects prokaryotes and eukaryotes in variety of ways, including growth, gene expression, physiology, stress resistance and metabolism reactions[13, 33–36]. For example, a previous study have found that microgravity stimulates the growth of *E. coli*, with a shortened lag phase, an increased exponential growth phase and the doubled final cell population density[37]. The simulated microgravity can also affect the production of microcin B17 by *E. coli* and the production of rapamycin by *Streptomyces hygroscopicus*[38, 39]. The eukaryotic *Saccharomyces cerevisiae* was reported to change gene expression in response to simulated microgravity[13, 40].

We suggested that hyper-gravity had a positive effect on total and active soil microbial community composition and diversity, with Shannon and Chao1 indices and microbial abundance were increased under the hyper-gravity conditions compared to that in the 1×g gravity. This may be a result of the stimulated proliferation of soil microorganisms under hyper-gravity conditions. Recent studies have also focused on the effects of hyper-gravity on the microbial proliferation except for survival. A study found that ultracentrifugation for a short time can induce soil microbial division to resist stress[41]. Similarly, a recent study revealed a variety of microorganisms, including Gram-negative *E. coli* and Gram-positive *Lactobacillus delbrueckii*. They can not only survive under hyper-gravity conditions but also display a robust proliferative behavior[8]. Moreover, they found microorganisms have different sensitivities that respond to hyper-gravity as they have species-specific biochemical processes[8]. On the other hand, microbial cells formed precipitated particles under hyper-gravity conditions, and the cell density increased with the increase of gravity[42]. Thus, the effects of sedimentation on the microbial communities can also explain the increase of microbial abundance and diversity under hyper-gravity conditions.

Besides, we found that the total soil microbial community separated from the active ones under different gravity conditions. Several studies investigated that the active microbial community from various environments such as sediments, soil and plankton using compared DNA with RNA sequencing methods[43–45]. The DNA based analysis does not provide information about the active microbial community and leads to an over-representation of several species in a community because it also contains dormant, inactive cells, as well as extracellular DNA[46]. In contrast, RNA is only stable in active cells, because potential extracellular RNA is rapidly degraded once cell death[47]. The rRNA abundance is proposed to be an index of potential activity, which represents the currently active microbial populations in environmental samples[48]. Thus, there were significant differences between total and active soil microbial communities obtained from DNA and RNA sequencing analysis, respectively. We also found that the composition of the total soil microbial community showed a greater response to hyper-gravity than active soil microbial community. It is difficult to distinguish and exclude the effect of extracellular DNA on the total microbial community, so total microbial community showed a complicated variation in the extreme environment. However, a recent study found that the total soil fungal community is significantly less sensitive to warming than the active fungal community[49]. We found that the absolute abundance of total microbial community showed a significant difference among hyper-gravity treatments but not in case of relative abundance. The absolute abundance of microbial 16S rRNA gene can better reflect the microbial composition and diversity than the relative abundance, because most bacteria possess different copy numbers of 16S rRNA gene[50].

For the coming new era of human expansion of the universe, such as future space travel to Mars, the exploration of microbiome in space can show us how many types of microbes can accumulate in such a unique environment. Recent study reveals that Martian soil samples are similar to those on Earth in physical, chemical features and microbial activity[51]. There is no situation in which humans carry microbes into outer space so far, and we speculate that outer space immigrants may not have infectious diseases caused by certain disease-causing bacteria and viruses. Once it exists, this microbe will have a great impact on the future in space. If the earthlings who landed on Mars later carried bacteria, then the whole Mars could be destroyed. So it is worthy to further study of infectious diseases under the condition of hyper-gravity.

Conclusions

Considering the data provided by our work, total and active soil microorganisms can survive and their community composition doesn’t change significantly under hyper-gravity conditions for 6 days. Future efforts to explore the maximum tolerance of soil total and active microorganisms or other species to hyper-gravity may be completed with the improvement of ultracentrifuges to achieve amazing gravity. Besides, our research provides a basis for exploring the effects of the vitality of life and evaluating the hypothesis that life may exist on other planets.

Declarations

Ethical approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

All authors have read and commented the manuscript and consent the publication.

Availability of data and materials

Not applicable

Competing interests

The author(s) declare no competing interest.

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Authors' contributions

LX contributed to the study design, DNA extraction, the data analyses and manuscript preparation. YL performed the data analysis, wrote and edited the ma the nuscript. KZ performed RNA extraction and laboratory analyses. SL performed the majority of bioinformatic analyses. ES and JX edited the manuscript. BM performed the study design and edited the manuscript. The authors read and approved the final manuscript.

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