Appl Microbiol Biotechnol
DOI 10.1007/s00253-016-8059-6

ENVIRONMENTAL BIOTECHNOLOGY

Simulated microgravity affects some biological characteristics of *Lactobacillus acidophilus*

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Received: 24 September 2016 / Revised: 1 December 2016 / Accepted: 7 December 2016

Abstract The effects of weightlessness on enteric microorganisms have been extensively studied, but have mainly been focused on pathogens. As a major component of the microbiome of the human intestinal tract, probiotics are important to keep the host healthy. Accordingly, understanding their changes under weightlessness conditions has substantial value. This study was carried out to investigate the characteristics of *Lactobacillus acidophilus*, a typical probiotic for humans, under simulated microgravity (SMG) conditions. The results revealed that SMG had no significant impact on the morphology of *L. acidophilus*, but markedly shortened its lag phase, enhanced its growth rate, acid tolerance ability up to pH < 2.5, and the bile resistance at the bile concentration of <0.05%. SMG also decreased the sensitivity of *L. acidophilus* to cefalexin, sulfur gentamicin, and sodium penicillin. No obvious effect of SMG was observed on the adhesion ability of *L. acidophilus* to Caco-2 cells. Moreover, after SMG treatment, both the culture of *L. acidophilus* and its liquid phase exhibited higher antibacterial activity against *S. typhimurium* and *S. aureus* in a time-dependent manner. The SMG treatment also increased the in vitro cholesterol-lowering ability of *L. acidophilus* by regulating the expression of the key cholesterol metabolism genes *CYP7A1, ABCB11, LDLR*, and *HMGCR* in the HepG2 cell line. Thus, the SMG treatment did have considerable influence on some biological activities and characteristics of *L. acidophilus* related to human health. These findings provided valuable information for understanding the influence of probiotics on human health under simulated microgravity conditions, at least.

Keywords Simulated microgravity · Probiotics · Characteristics · *Lactobacillus acidophilus* · Cholesterol-lowering

Introduction

Long term spaceflight is becoming an inevitable reality. How to protect the health of human during spaceflight is one of the urgent issues to be addressed. It has been widely recognized that environmental stresses would induce microorganisms to adapt their physiological, metabolic, and/or genetic variance for the corresponding environments. Such adaptation is also likely to occur under spaceflight conditions.

Previous studies indicated that the characteristics of microorganisms are greatly affected by either actual microgravity or simulated microgravity (SMG) which achieved through some ground-based systems, such as high-aspect rotating vessels (HARVs) and clinostats. It was deserved to refer that the SMG models have been demonstrated to be effective and been widely used by scientists to simulate the actual microgravity or reduced gravity conditions, because several studies have shown that results from various model systems are similar to results found in real microgravity (Brown et al. 2002; Klaus and Howard 2006). Experiments conducted in microbial liquid cultures under either actual microgravity or SMG conditions revealed a shorter lag phase, as well as prolonged log phase (Kacena et al. 1999), larger than average final cell population (Brown et al. 2002), modification of the production of

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Published online: 24 December 2016

secondary metabolites (Fang et al. 1997), and enhancement of biofilm formation (McLean et al. 2001). In addition, bacteria grown under these conditions exhibit resistance to multiple antimicrobial treatments (Lynch et al. 2004; Lynch et al. 2006; Rosenzweig et al. 2010), and becomes more virulent and thus could potentially cause damage to human health (Chopra et al. 2006; Nickerson et al. 2000). Transcriptome and proteome analyses were also carried out to reveal the mechanism of the effects of the actual microgravity or SMG on a wide variety of responses (Chopra et al. 2006; Crabbé et al. 2011; Vukanti et al. 2008).

For the studies about the effect of microgravity or SMG on intestinal microorganisms, the related reports to date were generally conducted on normal pathogens, such as *Escherichia coli, Staphylococcus aureus and Salmonellae* (Chopra et al. 2006; Nickerson et al. 2000; Rosado et al. 2010). Very few data are available showing the effect of microgravity on the behavior of probiotics, except for some studies indicating the significant decrease of the number of *Bifidobacterium* and *Lactobacillus* under microgravity conditions (Lizko 1991). More importantly, the decrease of these probiotics was accompanied by an increase of some pathogenic bacteria, such as *Escherichia coli* and *Proteus spp.* (Lizko 1991). Some probiotic strains possess characteristics, such as resistance tolerance to gastric acidity and bile toxicity, antibiotic resistance, adhesion to intestinal epithelial cells, inhibitory activity towards the growth of pathogenic bacteria, and inhibition of the adhesion of pathogenic bacteria (Soccol et al. 2010). These characteristics make such probiotics beneficial for the health of the host by improving the intestinal microbial balance and/or the metabolic activities of the indigenous flora (Darilmaz et al. 2011). Whether the decrease of probiotic bacteria under microgravity conditions is due to the deterioration of survival performance, or the increased cell population or/and the virulence of some pathogenic microorganisms, remains to be explored. In addition, ecological regulation using additive probiotics was thought to be helpful for the regulation and balance of gut flora. However, it is still unknown whether this kind of regulatory approach under space environments would be effective. Accordingly, there is a great interest in understanding the effects of microgravity on the performance of these probiotics.

Lactobacillus acidophilus (*L. acidophilus*) has been extensively reported as a major beneficial component of the microbiota in the human tract and is widely used as probiotics for ecological regulation in normal life on Earth. Little is known about what will happen when this strain lives under microgravity conditions. In this study, the characteristics of *L. acidophilus* under simulated microgravity conditions were primarily investigated focusing on the following aspects: (1) growth curve and rate, acid and bile tolerance, antibiotic susceptibility, and the ability of the *L. acidophilus* cells to adhere to Caco-2 cells and (2) antimicrobial property and cholesterol-lowering ability in HepG2 cells of the cultured medium (with and/or without cells). The obtained results are expected to contribute some basic information on the responses of probiotics to the simulated microgravity conditions at the least.

Materials and methods

Bacteria strain and culture conditions

L. acidophilus (ATCC 4356) was bought from the Culture Collection Center of the Chinese Academy of Sciences (Beijing, China) and used throughout this study.

Escherichia coli (*E. coli* ATCC 25922), *Salmonella enteric serovar typhimurium* (*S. typhimurium* CMCC (B) 50,115) and *Staphylococcus aureus* (*S. aureus* ATCC 25923) were maintained in the School of Life Sciences, Northwestern Polytechnical University (Xi'an, China).

Cell lines and cell cultures

The human Caco-2 and HepG2 cells were gifts from the Fourth Military Medical University (Xi'an, China) and were grown as monolayers in complete Dulbecco's modified Eagle's medium (DMEM) supplemented with 20% fetal bovine serum (FBS), penicillin, and streptomycin (100 μg/ml) in a 5% CO_2 atmosphere at 37 °C. For subcultures, the cells were harvested by digesting with 0.25% trypsin/0.02% EDTA and resuspended in fresh DMEM media.

Exposure of *L. acidophilus* to simulated microgravity (SMG)

A 2-D RWV (developed by the Chinese Astronaut Research and Training Center, Fig. 1) was utilized for simulating

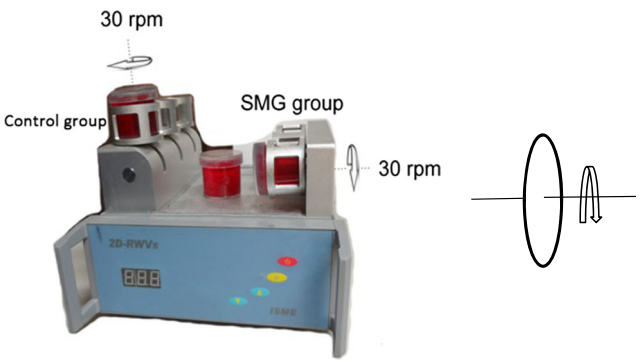

Fig. 1 The 2-D RWV SMG system. The clinostat is equipped with computerized temperature and motor controls. Cells were initially seeded into the chambers. The chambers were filled with MRS medium, and air bubbles were carefully removed. The chambers rotated around a horizontal axis at 30 rpm to achieve a time-averaged gravity vector of 10^{-3} g (SMG-treated group), that the cells cannot feel gravity, so that has effects similar to actual weightlessness. The cells grown in rotated chambers around a vertical axis served as control (non-SMG-treated group)

microgravity conditions. The clinostat is a device in which samples are rotated to prevent the biological system from perceiving the gravitational acceleration vector. The chambers rotated around a horizontal axis at 30 rpm to achieve a time-averaged gravity vector of 10^{-3} g (SMG-treated group), that the cells cannot feel gravity, so that has effects similar to actual weightlessness. The cells grown in vertically rotated chambers served as control (non-SMG-treated group) to exclude the effects of vibration. The shear stress was limited by filling the chamber completely with the culture medium (Shi et al. 2012).

Exposure of *L. acidophilus* to SMG: After adjusting the OD_{600} value to 0.03 or 0.3 according to the experimental design, the *L. acidophilus* cells were transferred to the above mentioned chambers to set up the control and SMG groups. The SMG and control cultures were simultaneously grown under the conditions of 30 rpm and 37 °C for the designed period.

Morphological analysis of *L. Acidophilus* by scanning electron microscopy

Scanning Electron Microscopy (SEM), with a JSM-6360LV scanning electron microscope (Jeol, Tokyo, Japan), was used to observe the morphology of *L. acidophilus*. Briefly, the 24 h treated bacteria were centrifuged at 4000 rpm for 15 min to collect the cells. The bacterial pellet was resuspended in a 2.5% glutaraldehyde solution and kept for 2 h to fix the cells. The fixed bacterial cells were washed with 0.1 M phosphate buffer and centrifuged at 3000 rpm for 5 mins, and then the supernatant was discarded. After another wash, the obtained cells were dehydrated in an ethanol gradient at concentrations of 30, 50, 70, 80, 90, and 100% in that order, for 20 min in each solution. Next, the dehydrated cells were treated with TBA (tert-butyl alcohol) for 30 mins to remove the residual ethanol and collected by centrifugation at 3000 rpm for 5 mins. After repeating the TBA treatment three times, the cells were suspended in 100 μl TBA, dropped on a mica plate, air dried at room temperature, then coated in gold, and finally observed by SEM.

Investigation of the profiles of bacterial growth

The profile of the bacterial growth was investigated by the viable plate counting method. Briefly, after adjusting the initial OD600 value of the inoculated bacteria culture to about 0.03, the number of bacteria was counted in MRS agar plates by using the serial dilution method and shown as log CFU/ml. The cultivation was carried out under the conditions indicated as control and SMG environment, separately, as described in Section 2.3. During the cultivation, the amount of the bacteria was recorded at 2- or 4-h intervals, up to 30 h. The growth rate was expressed as the mean value of the change of the number of bacteria per hour.

Measurement of the acid tolerance

L. acidophilus was tested for their ability to survive under acidic conditions by using the methods described by Nickerson et al. (2000) with some modifications. The bacteria cultures were separately inoculated in the fresh de Man, Rogosa and Sharpe (MRS) medium at pH 1.5, 2.5, 3.5, 4.5, and the original pH of 6.2. After adjusting the OD_{600} value of the liquid culture to 0.3, the amount of the bacteria (the original amount) was counted in MRS agar plates using the serial dilution method. The counted bacteria cultures were then cultivated at 37 °C under the designed control or SMG environments as described in Section 2.3. During the cultivation, the number of bacteria was counted at 6 h, and the survival rate was calculated accordingly.

Measurement of bile salt tolerance

The bile salt toxicity tolerance analysis was conducted under the above mentioned conditions and methods, except that the medium contained 0.03, 0.05, 0.1, 0.3, 0.5% of bile (oxgall, Sigma-Aldrich, Saint Louis, MO, SUA) and the pH was the natural pH (6.2).

The assay of antibiotic sensitivity

For detection of the antibiotic sensitivity of *L. acidophilus*, the cultivation was carried out under the same conditions as above except that the MRS medium without oxgall was additionally supplemented with 10 μg/mL cefalexin, 2000 μg/mL chloramphenicol, 20 μg/mL sulfur gentamicin, or 12 μg/mL sodium penicillin. All the cultures were incubated at 37 °C for 18 h. At the end of cultivation, the number of bacteria was determined by the serial dilution method, and the survival data was calculated accordingly.

Analysis of the adhesive ability of the bacteria to Caco-2 cells

The adhesive ability of the *L. acidophilus* strain to the Caco-2 cells was determined as previously described by Chopra et al. (2006) with minor modifications. The Caco-2 cells were suspended at a density of 5×10^5 cells/mL, and cultured in a 24-well plate until the cell monolayer reached confluence. A 100 μL volume of the 24-h-SMG-treated or nontreated *L. acidophilus* suspension was added to the DMEM medium-washed Caco-2 cells at a concentration of 5×10^7 CFU/hole (counted by hemocytometer). A 900 μL volume of fresh DMEM medium (without antibiotics) was also added into the mixture of bacteria and Caco-2 cells and

incubated at 37 °C for 2 h. Subsequently, the mixture was washed five times with PBS to remove the non-adherent bacteria from the Caco-2 cells. After adding 0.5 mL PBS containing 0.05% (*v*/v) Triton X-100, the cells were lysed for 30 min at 37 °C. Then, the cell lysates together with bacteria were serially diluted. A 100 μL volume of the dilution was inoculated onto MRS agar plates. After incubation for 48 h at 37 °C, the number of adhered bacteria was counted. The adhesion rate was expressed as the percentage of the number of adherent bacteria to the original number of bacteria.

The antibacterial activity of the *L. acidophilus* culture

Antimicrobial activity of the L. acidophilus bacterial culture suspension and its liquid phase (supernatant) against different pathogens

The antimicrobial activities of the bacterial culture suspension and the liquid phase of the 24 h treated or non-treated *L. acidophilus* against *E. coli*, *S. typhimurium* and *S. aureus* were separately measured. The supernatant of the culture was obtained after a centrifugation at 2000×*g* at 4 °C for 10 min using a 5804 R centrifuge (Eppendorf AG 22331, Hamburg, Germany). The disk diffusion method was used with minor modifications (Min et al. 2008). Briefly, a 100 μl inoculum of *E. coli*, *S. typhimurium*, and *S. aureus* (10^6 CFU/mL) was spread onto LB agar plates. Disks (diameter = 8 mm) were saturated with the bacterial suspension or the liquid phase of the *L. acidophilus* culture and aseptically placed on the above LB agar media. After incubation for 24 h at 37 °C, the diameter of the inhibition zone was determined according to the cross method using a Vernier caliper (HMCT Group, Harbin, China).

Antimicrobial activity of the supernatant of L. acidophilus culture obtained at different cultivation times against S. typhimurium

The antimicrobial activity of the liquid phase of the SMG treated and non-treated cultures of *L. acidophilus* against *S. typhimurium* was measured according to the above methods, with supernatant collected at different cultivation times (6, 12, 20, 24, and 36 h).

Cholesterol-lowering ability

CCK8 assay for cell viability

HepG2 cells (1×10^4/well) were seeded in 96-well plates and separately treated with 1, 1/2, 1/4, 1/8, and 1/16 concentration of the liquid phase of the SMG-treated and non-treated cultures of *L. acidophilus*. Fresh MRS medium was also tested as a control. The treatment was carried out for 48 h at 37 °C in a 5% CO_2 incubator. Subsequently, a 20 μL volume of CCK-8 assay reagent (Cell Counting Kit-8, Dojindo, Japan) was added to a 200 μL cell suspension per well in the 96-well plate in triplicate for each treatment. After incubation for another 2 h, the OD value at 450 nm of each well was read by a microplate reader (BioTek Synergy-4). The cell viability was expressed as the percentage of the absorbance values of the treatment group to that of the control group, which was considered as 100% (Li et al. 2014).

Analysis of cellular total cholesterol (TC) and extracellular bile acid contents

The measurements were performed using enzyme assay kits (APPLUGEN, Beijing, China). HepG2 cells were seeded in a 75 cm^2 cell culture flask at a density of 6×10^5 cells per flask and treated with the 1/8 concentration of the liquid phase of *L. acidophilus* culture. After a treatment for 48 h, the liquid phase of the cell culture was collected to measure the level of extracellular bile acid content, and the cells were washed three times with PBS and collected to determine the cellular TC content.

Analysis on the mechanisms of cholesterol-lowering ability

RT-PCR was used to measure the levels of mRNA expressions of key genes related to cholesterol metabolism in cells. The tested genes were cholesterol 7a–hydroxylase (*CYP7A1*), bile salt export pump (*BSEP*), hydroxy-3-methylglutaryl-CoA reductase (*HMGCR*), and the LDL receptor (*LDLR*).

The primers used in the study are listed in Table 1. The total RNA from HepG2 cells was extracted using the TRIzol plus RNA purification kit (Invitrogen, Carlsbad, CA). Synthesis and amplification of the cDNA were carried out using a High Capacity cDNA Reverse Transcription Kit (TransGen Biotech, Beijing, China) following the manufacturer's instructions. RT-PCR was performed in 96-well plates on an ABI qRT-PCR 7500 system (Applied Biosystems, Foster City,

Table 1 Primer sequences used for RT-PCR analysis in the study

Primers		Sequences (5′ to 3′)
β-actin	Forward	AGCCATGTACGTAGCCATCC
	Reverse	CTCTCAGCTGTGGTGGTGAA
CYP7A1	Forward	ACTACCTTCTATTGTAATGGACTC
	Reverse	TTCAATATCATTCAATGGCGGC
ABCB11	Forward	TTCATTGAAGCACTTGAGACTGAG
	Reverse	TTGCACTCAGTACAACTGCAGAGATC
HMGCR	Forward	ACATTGTCACCGCCATCTACATTGC
	Reverse	GGCTTGCTGAGGTAGTAGGTTGCT
LDLR	Forward	CTGTCTCTGTTGCGGATACCAAGG
	Reverse	GCGAGTAGATGTCCACACCATTCA

CA) using SYBR (TaKaRa) as the fluorophore. Amplifications were performed under the following conditions: 94 °C for 2 min, 35 cycles of 94 °C for 20 s, 55 °C for 20 s, and 72 °C for 30 s. At the end of the amplification cycle, a melting analysis was conducted to verify the specificity of the reaction. Differences in mRNA expression were calculated using the $^{\triangle\triangle}$CT method after normalizing to β-actin expression (Shao et al. 2013).

Statistical analysis

All experiments were carried out in triplicate and the mean values are reported. The Duncan ($\alpha = 0.05$) test was performed using the SPSS software (Ver.17.0, SPSS Inc., Chicago, IL, USA), and the statistical differences between treatments were determined.

Results

Cell morphology

The morphology and surface microstructure of non-SMG-treated and SMG- treated *L. acidophilus* are shown in Fig. 2a, b, respectively. The two groups showed similar morphological and structural features, indicating that 1-day-SMG-treatment had no significant effect on the morphology and structure of *L. acidophilus*.

Growth profile and growth rate

The effect of SMG on the growth profile and growth rate of *L. acidophilus* is shown in Fig. 3a and b, respectively. Compared to the control group, the SMG treatment resulted in a shorter lag phase, a faster growth during the first 6 h and a higher growth rate at the significance level of $P < 0.05$. However, after 6 h, the control showed an increased growth rate and reached a similar number of cells as the SMG treated group by 16 h. This indicated that SMG could only accelerate the cell growth at the early stages, but not change their end level.

Tolerance to acid

As the results in Fig. 4 show, at higher acidic conditions (pH < 2.5), the SMG treatment significantly improved the survival of *L. acidophilus* ($P < 0.05$). As indicated, at the pH of 1.5 and 2.5, the survival rate was significantly increased from 25.94 and 68.35% in the non-SMG-treated group to 57.71 and 90.85% of the treated groups, respectively, ($P < 0.05$). However, at higher pH, no significant effects on the SMG-treated group were observed compared to the control group.

Tolerance to bile toxicity

The effect of SMG on the tolerance to bile by *L. acidophilus* was measured with 0.03, 0.05, 0.1, 0.2, and 0.3% bile (Oxgall) (Fig. 5). It is evident that the SMG treatment was able to significantly improve the tolerance to bile toxicity of *L. acidophilus* at low concentrations of bile (<0.05%), but not at high concentrations of bile (0.1%–0.3%).

Antibiotic sensitivity

The effect of SMG on the antibiotic susceptibility of *L. acidophilus* to cefalexin, chloramphenicol, sulfur gentamicin, and sodium penicillin is shown in Fig. 6. Except for chloramphenicol, the SMG treatment caused a significant enhancement of cell tolerance to the other antibiotics tested ($P < 0.05$). Especially for sulfur gentamicin, the cell survival rate was increased from 271.58 to 970.83% after the SMG treatment.

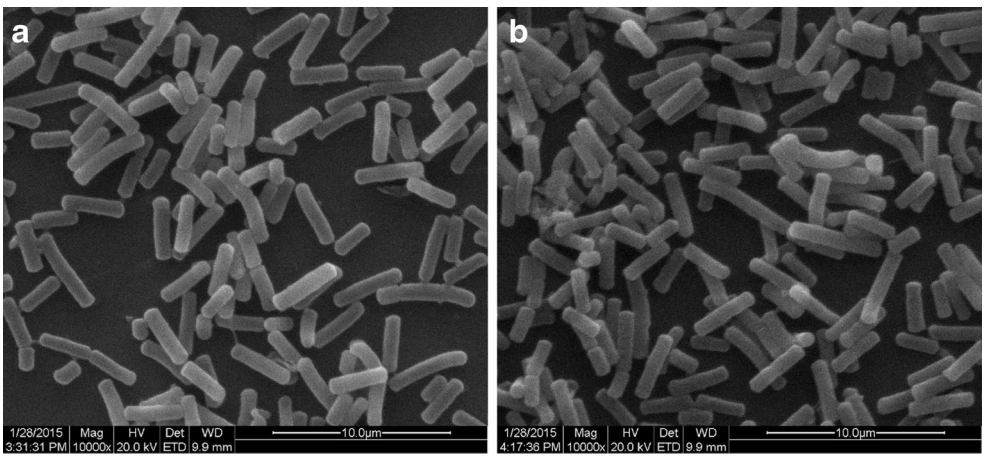

Fig. 2 Effect of simulated microgravity (SMG) on the morphology of *L. acidophilus*. **a** non-SMG-treated. **b** SMG-treated

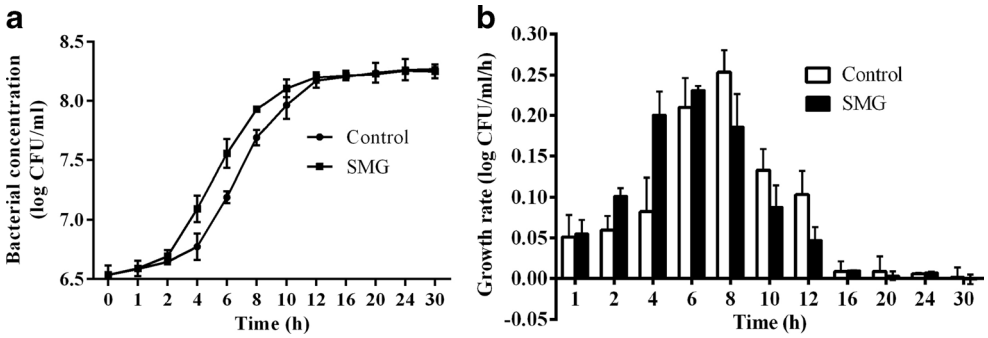

Fig. 3 Effect of simulated microgravity (SMG) on the growth curve (**a**) and growth rate (**b**) of *L. acidophilus*

The adhesive ability to Caco-2 cells

The SMG treatment had no statistically significant influence on the ability of *L. acidophilus* to adhere to Caco-2 cells, although the corresponding value was decreased from 4.32 to 3.64% in the SMG group ($P > 0.05$) (Fig. 7).

Antibacterial activity

The inhibition of the different bacterial pathogens cultures with bacterial suspension and liquid phase of *L. acidophilus* are shown in Fig. 8a and b. The results showed that none of the samples had inhibitory activity against *E. coli* (data no shown), but all showed a strong inhibitory activity against *S. typhimurium* and *S. aureus*. It should be noted that the bacterial cell suspension had higher inhibitory activity against the pathogens than the supernatant only ($P < 0.05$). After SMG treatments, the antibacterial activity against *S. typhimurium* and *S. aureus* was significantly increased for all samples ($P < 0.05$). Additionally, the SMG treatment also increased the antibacterial activity of *L. acidophilus* in a time-dependent manner (Fig. 8c). Taking the antimicrobial ability against *S. typhimurium* as example, compared to the non-SMG-treated group, the SMG effect was not significant at 6–12 h ($P > 0.05$), but markedly increased at 20–24 h ($P < 0.05$). However, the difference disappeared at the end of the incubation (36 h).

Cholesterol-lowering function

Cell viability

There was no statistically significant difference between the SMG and control groups of *L. acidophilus* regarding their effect on the growth of HepG2 cells at all tested concentrations ($P > 0.05$), as shown in Fig. 9a. Comparatively, 1/8 of the original liquid phase of the *L. acidophilus* culture was used in further studies to avoid the excessive cytotoxicity to the cells.

Determination of cellular TC and extracellular bile acid contents

The liquid phase of both the SMG-treated and non-SMG-treated *L. acidophilus* significantly lowered the TC content of HepG2 cells ($P < 0.05$), as shown in Fig. 9b. Comparatively, the SMG-treated group had higher cholesterol-lowering ability than the non-SMG-treated group ($P < 0.05$), corresponding to a reduction of TC content of 53.04 ± 21.74 and 30.48 ± 8.14%, respectively. The amount of bile acid secreted to the medium was also significantly increased by 32.82 ± 5.47% by the control group and 46.52 ± 10.60% by the SMG group ($P < 0.05$) (Fig. 9c).

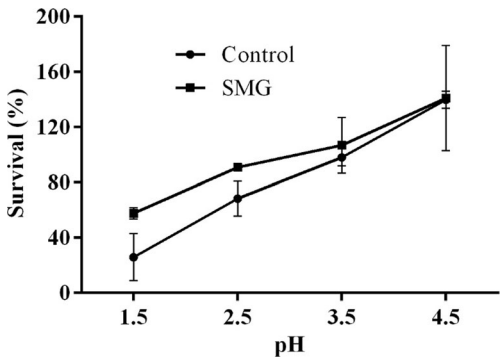

Fig. 4 Effect of simulated microgravity (SMG) on the survival of *L. acidophilus* under different pH

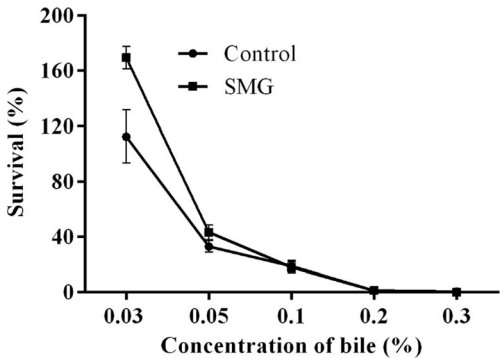

Fig. 5 Effect of simulated microgravity (SMG) on the survival of *L. acidophilus* under different concentrations of bile

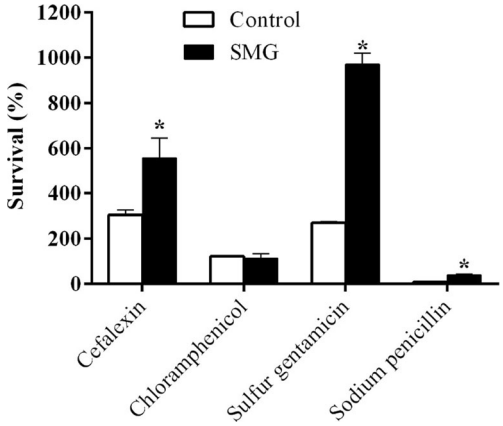

Fig. 6 Effect of simulated microgravity (SMG) on the survival of *L. acidophilus* exposed to different antibiotics. *Asterisk* indicates significant difference at $P < 0.05$

The level of mRNA expressions of key genes related to cholesterol metabolism

The mRNA expression levels of key genes related to cholesterol metabolism in HepG2 cells were analyzed to determine the cholesterol-lowering mechanism of the liquid phase of the *L. acidophilus* culture. As shown in Fig. 9d, The liquid cultures from both the non-SMG-treated and SMG-treated groups increased the expression of the following genes: *CYP7A1* (a key gene in the biosynthesis of bile acid) by 1.66-fold and 2.23-fold ($P < 0.05$), respectively; *ABCB11* (a crucial gene involved in the efflux of bile acid) was elevated by 1.60- and 1.53-fold, respectively; *LDLR* (a key gene in the absorption of plasma LDL-C into the liver) was raised by 2.22- and 3.22-fold ($P < 0.05$), respectively. In contrast, the expression of *HMGCR* (an essential gene in the de novo synthesis of cholesterol) was decreased to 0.92 ($P > 0.05$) and 0.31 times ($P < 0.05$) of the control by the cultures of non-SMG-treated and SMG-treated groups, respectively.

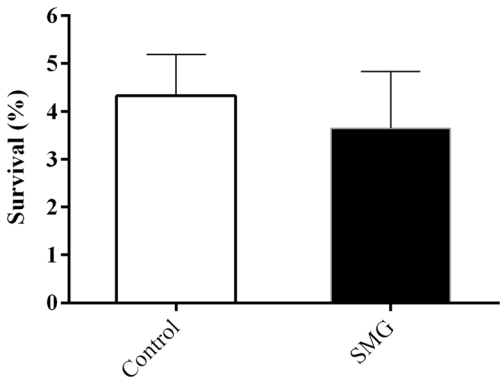

Fig. 7 Effect of simulated microgravity (SMG) on the adhesion ability of *L. acidophilus* to Caco-2 cells

Discussion

Along with the development of manned space technology, long-term spaceflights have been extensively discussed and planned. It is necessary to specifically determine the effect of exposure to harsh environmental conditions in space, such as microgravity, vacuum, and radiation on the gut microbiota (Edsall and Franzodendaal 2014; Mermel 2013). Previous studies on the effect of microgravity or SMG on intestinal microorganisms were generally focused on human pathogenic microorganisms. However, the effect of microgravity on the characteristics of probiotics, one of the most important factors in the fight against human pathogens, has not been extensively studied up to now.

This study revealed that the 1-day-SMG treatment did not have a significant effect on the morphology of *L. acidophilus*, but it greatly shortened its lag phase and enhanced its growth rate. This might be due to the SMG conditions that leads to the absence of sedimentation and buoyancy-driven convection in the environment, which increases the availability of oxygen and nutrients from the surrounding environment, thus improving the efficiency of substrate utilization by the cultured bacteria (Brown et al. 2002). The disappearance of this effect at the later stage of bacteria growth might be due to the nutrient limit at that time.

The ability to tolerate the stressful conditions of the stomach and upper intestine that contain bile is also important for lactobacilli to exert its positive health effects (Chou and Weimer 1999). In the present study, the SMG treatment was found to significantly contribute to improve the survival rate of *L. acidophilus* at low pH levels (pH < 2.5), but not at higher pH levels. This might be due to the inability of the bacteria to grow well enough and the positive effect of the SMG treatment was not so significant.

Previous studies suggested that the common bile resistance mechanism in lactic acid bacteria was strongly connected to the presence of bile salt hydrolase activity, which probably exerts a detoxification effect by catalyzing the hydrolysis of glycine or taurine-conjugated bile salts into amino acid residues and unconjugated bile salts (Yin et al. 2011). The SMG treatment might improve the activity of the related hydrolase and increase the survival rate of the cells at the low bile concentration (<0.05%). However, the increase of the enzyme activity by the SMG treatment was not strong enough to cause more significant detoxification effect at high bile concentration levels. As a result, no marked difference was observed in the survival rate of the cells between the SMG and non-SMG-treated groups.

During space travel, there is a dysregulation of the human immune system which may increase risk of infection (Crucian et al. 2008). In addition, anaerobic colonic flora is diminished with a proportionate increase in aerobic bacteria such as *Pseudomonas* and *S. aureus* (Ilyin 2005; Mermel 2013).

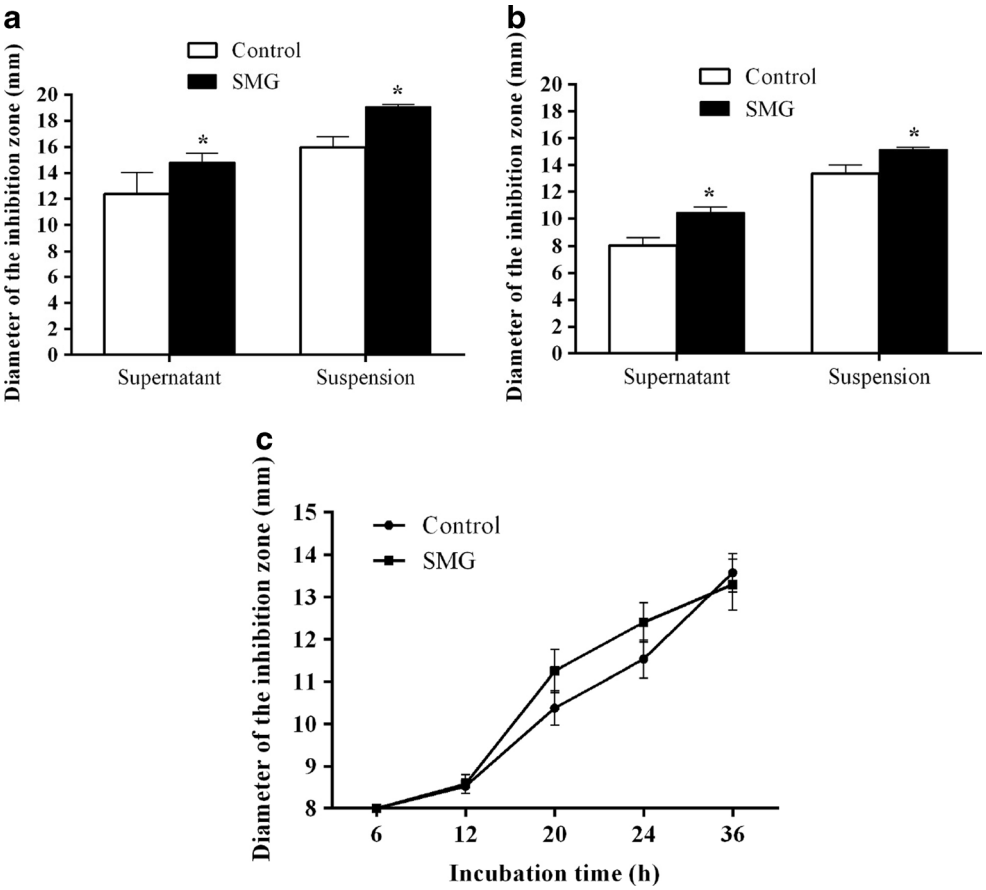

Fig. 8 Effect of simulated microgravity (SMG) on the antibacterial activity of the supernatant and bacterial suspension against *S. typhimurium* (**a**) and *S. aureus* (**b**) of the *L. acidophilus* and the inhibition activity of the supernatants with different incubation times against to *S. typhimurium* (**c**). *Asterisk* indicates significant difference at $P < 0.05$

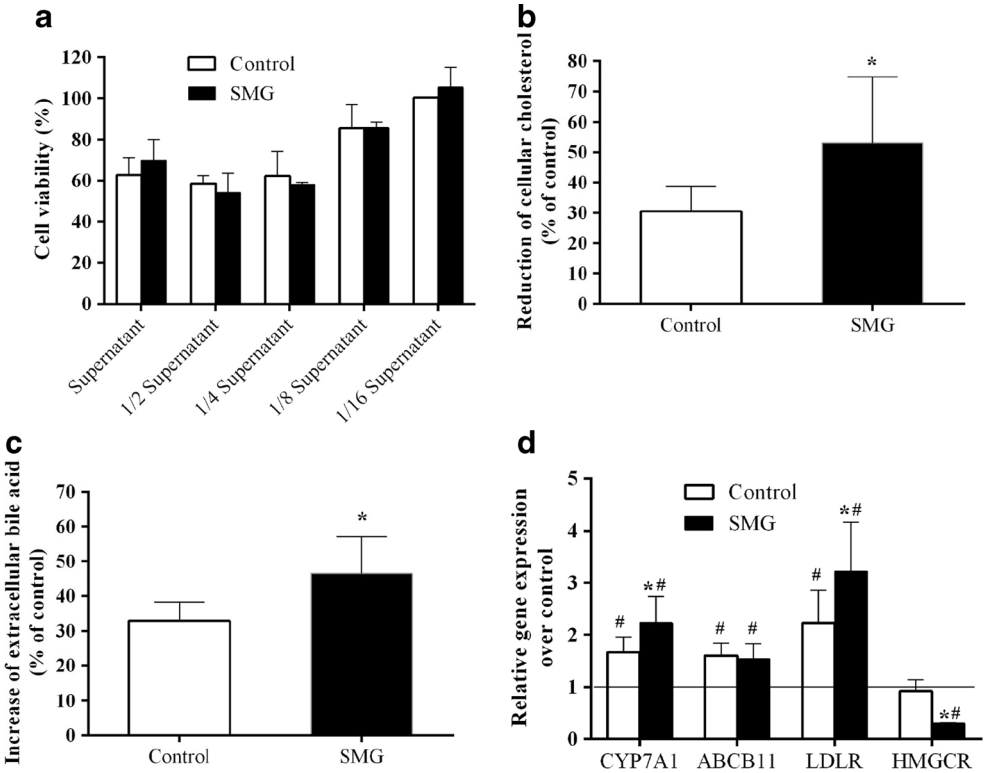

Fig. 9 Effect of simulated microgravity (SMG) on the cholesterol-lowering activity of the supernatant of the *L. acidophilus* **a** cell viability, **b** reduction of cellular total cholesterol content, **c** increase of extracellular bile acid content, and **d** gene expression related to cholesterol metabolism. *Asterisk* indicates significant difference between non-SMG-treated and SMG-treated at $P < 0.05$. *Sharp* represents significant difference from control at $P < 0.05$

There is also a greater abundance of *S. aureus*, along with *Enterobacteriaceae* (Mermel 2013), which may cause enteric dysbacteriosis. Accordingly, the antibiotic therapy is a common and rational approach to treat these infections or gastrointestinal disorders. Previous studies showed that some pathogens such as *E.coli* could exhibit resistance to multiple antimicrobial treatments under either actual reduced gravity or SMG conditions (Lynch et al. 2006; Taylor and Sommer 2005) which was potentially bad for therapy. The present study showed that the sensitivity of *L. acidophilus* to most of the tested antibiotics was also lowered under the SMG conditions, which might ensure that the intestinal probiotics are not easily destroyed by antibiotics, and thus would be beneficial for maintaining the host health, to a certain extent.

The ability of probiotic bacteria to adhere to the intestinal epithelium is a prerequisite for probiotic microorganisms to be effective. Therefore, the ability to adhere to epithelial cells and mucosal surfaces is an important property of many probiotic bacterial strains (Collado et al. 2005; Ouwehand et al. 1999). The SMG conditions were reported to increase the invasive potential of *Salmonella enteric serovar typhimurium* (Chopra et al. 2006). However, in this study, no statistically change was detected when the SMG treatment was used for *L. acidophilus*, although slightly lower adhesion ability of *L. acidophilus* to Caco-2 cells was found in the SMG-treated group than in the control group.

It is noted that when under the SMG conditions, lack of sedimentation results in uniform cell distribution throughout the clinostat, which means the bacteria have higher accessibility to the undesirable cultural environments such as higher acid and bile salt concentration and some antibiotics. However, the tested bacteria showed stronger resistant ability to the aforementioned bad environments when under the SMG conditions. It indicated that the bacteria itself may have made some changes to adapt the changed environments or more resistant substances were produced because of the more accessibility to the nutrients of the bacteria, against the disadvantageous environments. It was ever reported that the *E.coli* grown under SMG conditions exhibited enhance ability to survive sub-lethal doses of antimicrobial agents by increasing its membrane potential and integrity (Vukanti, et al. 2012). SMG conditions had also been found to markedly increase the production of the enterotoxigenic *Escherichia coli* (ETEC) heat-labile enterotoxin, which induced fluid secretory responses in a mouse model (Chopra et al. 2006). Therefore, further studies such as transcriptome and proteome analyses are needed to examine the changes of the bacteria itself and more importantly, the mechanisms of the SMG influencing the bacteria which is still unknown are urgently need to be investigated.

As a functional probiotic, the anti-pathogen activity is one of important properties of *L. acidophilus*. The antimicrobial ability of *L. acidophilus* against some enteropathogens was analyzed in this study. The bacterial suspension showed relative stronger inhibition activity against two tested pathogens than the liquid phase of the culture. This illustrated that the bacterial cells also had inhibitory ability against the enteropathogens. More importantly, the SMG-treated cultures always exhibited higher antibacterial activity than those of control. The antimicrobial activity of lactic acid bacteria against pathogens could be due to their production of organic acids, hydrogen peroxide, and bacteriocins (Vesterlund 2009). Therefore, it is generally possible that the SMG treatment could induce the production of some bactericidal or bacteriostatic metabolites, such as bacteriocins by *L. acidophilus*. However, further investigation is still needed to understand the mechanisms of this effect. Moreover, the SMG treatment exerted some influence on the inhibitory activity of *L. acidophilus* in a time-dependent manner. The production of antibacterial components was not significantly increased by the SMG treatment in the early stage of cultivation, but it was elevated in the later cultivation stages. Finally, an increase of antibacterial activity by the SMG treatment was detected after incubation for 20–24 h. However, this increased activity gradually disappeared that may be due to nutrient exhaustion in the end stage.

It has been reported that *L. acidophilus* (ATCC 4356) can secrete soluble molecules that inhibit the intake of cholesterol by Caco-2 cells by downregulating the expression of NPCL1(Huang and Zheng 2010). Liver is the major organ where the cholesterol metabolism occurs. In liver, cholesterol is mainly converted into bile acids, and then bile acids are excreted into the intestine (Meier and Stieger 2002). The more synthesis and efflux of bile acids, the more cholesterol is eliminated. In the present study, this was confirmed by the observed reduction of cellular total cholesterol content and the increase of extracellular bile acid content in all the tested samples. In addition, it was found in this study that the liquid phase of *L. acidophilus* culture reduced the hepatic cholesterol content by upregulating the expression of genes involved in bile acid synthesis (*CYP7A1*) and efflux (*ABCB11*) and by downregulating the gene responsible for the de novo synthesis of cholesterol (*HMGCR*). The SMG treatment exerted greater influence in this way. The significant upregulation of *CYP7A1* in the non-SMG-treated and SMG-treated groups indicated that the bile acid synthesis was significantly increased by the SMG treatment. Comparable with the upregulation of *CYP7A1*, the expression of *ABCB11* was also significantly increased in both non-SMG and SMG-treated groups. This would lead to a significant increase of the efflux of bile acids (Wang et al. 2012), which in turn results in a feedback signaling to promote more conversion of cholesterol to bile acids and, thus lowered the hepatic cholesterol level. Furthermore, the significant reduction in *HMGCR* expression of SMG-treated groups inhibited the de novo synthesis of cholesterol, and thus decreased its accumulation in the liver. The overall decrease in the hepatic cholesterol content mentioned above increases the demand for cholesterol in liver and would trigger

the upregulation of *LDLR*, which would promote more LDL-C absorption from plasma to liver, and then could improve the clearance of plasma LDL-C if an animal model was used (Bartley et al. 2010). This is the first time that the cholesterol-lowering mechanism of *L. acidophilus* in view of hepatic cholesterol metabolism was elucidated, although similar observations have been made by other researchers using other probiotics. This is also the first time that the impact of probiotics on cholesterol metabolism under SMG condition is revealed. There have been reports that the International Space Station (ISS) strains produced larger amounts of exopolymeric substances than similar control strains on Earth (Mauclaire and Egli 2010). Therefore, it is conceivable that the SMG treatment would induce the secretion of more effective molecules or new functional components produced by *L. acidophilus* and thus eventually further reduce cholesterol content. Additional research is still needed to understand the mechanisms involved in this phenomenon.

In conclusion, the present study revealed that the simulated microgravity conditions can significantly impact the biological characteristics of the tested *L. acidophilus*, in various aspects, including growth profile, acid and bile tolerance, antibiotic susceptibility of the bacterial cells, and the antimicrobial property and ability of lowering cholesterol in HepG2 cells in the cultured medium. However, the ability of the bacteria to adhere to Caco-2 cells was not significantly changed under SMG conditions. Overall, our study provides essential information for better understanding the influence of simulated microgravity on probiotic bacteria, and thus may contribute to ensure the health of the astronauts in future space flights.

Acknowledgements This study was funded by the project of National Natural Science Funding (No. 31471718), National Key Technology R&D Program (No. 2015BAD16B02), the Agriculture Department of China (No. CARS-30), Shaanxi Provincial Natural Science Foundation (No. 2015JQ3083), Fundamental Research Funds for the Central Universities (No. 3102016QD075 and 3102014JCQ15001), and the Foundation of He'nan Educational Committee (17B550005).

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

Conflict of interest The authors declare that they have no conflict of interest.

References

Bartley GE, Yokoyama W, Young SA, Anderson WHK, Hung SC, Albers DR, Langhorst ML, Kim H (2010) Hypocholesterolemic effects of hydroxypropyl methylcellulose are mediated by altered gene expression in hepatic bile and cholesterol pathways of male hamsters. J Nutr 140:1255–1260. doi:10.3945/jn.109.118349

Brown R, Klaus D, Todd P (2002) Effects of space flight, clinorotation, and centrifugation on the substrate utilization efficiency of *E. coli*. Microgravity Sci Tec 13:24–29

Chopra V, Fadl A, Sha J, Chopra S, Galindo C, Chopra A (2006) Alterations in the virulence potential of enteric pathogens and bacterial–host cell interactions under simulated microgravity conditions. J Toxicol Env Heal A 69:1345–1370. doi:10.1080/15287390500361792

Chou LS, Weimer B (1999) Isolation and characterization of acid and biletolerant isolates from strains of *Lactobacillus acidophilus*. J Dairy Sci 82:23–31. doi:10.3168/jds.S0022-0302(99)75204-5

Collado MC, Gueimonde M, Hernandez M, Sanz Y, Salminen S (2005) Adhesion of selected *Bifidobacterium* strains to human intestinal mucus and its role in enteropathogen exclusion. J Food Protect 68:2672–2678

Crabbé A, Schurr MJ, Monsieurs P, Morici L, Schurr J, Wilson JW, Ott CM, Tsaprailis G, Pierson DL, Stefanyshyn-Piper H (2011) Transcriptional and proteomic responses of *Pseudomonas aeruginosa* PAO1 to spaceflight conditions involve Hfq regulation and reveal a role for oxygen. Appl Environ Microb 77:1221–1230. doi:10.1128/AEM.01582-10

Crucian B, Stowe R, Pierson D, Sams C (2008) Immune system dysregulation following short- vs long-duration spaceflight. Aviat Space Envir Md 79:835–843. doi:10.3357/ASEM.2276.2008

Darilmaz D, Ashm B, Suludere Z, Akca G (2011) Influence of gastrointestinal system conditions on adhesion of exopolysaccharide-producing *Lactobacillus delbrueckii subsp. bulgaricus* strains to Caco-2 cells. Braz Arch Biol Technol 54:917–926. doi:10.1590/S1516-89132011000500009

Edsall SC, Franzodendaal TA (2014) An assessment of the long-term effects of simulated microgravity on cranial neural crest cells in zebrafish embryos with a focus on the adult skeleton. PLoS One 9:e89296. doi:10.1371/journal.pone.0089296

Fang A, Pierson D, Koenig D, Mishra S, Demain A (1997) Effect of simulated microgravity and shear stress on microcin B17 production by *Escherichia coli* and its excretion into the medium. Appl Environ Microb 63:4090–4092

Huang Y, Zheng Y (2010) The probiotic *Lactobacillus acidophilus* reduces cholesterol absorption through the down-regulation of Niemann-pick C1-like 1 in Caco-2 cells. Brit. J Nutr 103:473–478. doi:10.1017/S0007114509991991

Ilyin V (2005) Microbiological status of cosmonauts during orbital spaceflights on Salyut and Mir orbital stations. Acta Astronaut 56:839–850. doi:10.1016/j.actaastro.2005.01.009

Kacena M, Merrell G, Manfredi B, Smith E, Klaus D, Todd P (1999) Bacterial growth in space flight: logistic growth curve parameters for *Escherichia coli* and *Bacillus subtilis*. Appl Microbiol Biot 51:229–234. doi:10.1007/s002530051386

Klaus DM, Howard HN (2006) Antibiotic efficacy and microbial virulence during space flight. Trends Biotechnol 24:131–136. doi:10.1016/j.tibtech.2006.01.008

Li Q, Huyan T, Ye LJ, Li J, Shi JL, Huang QS (2014) Concentration-dependent biphasic effects of resveratrol on human natural killer cells *in Vitro*. J Agric Food Chem 62:10928–10935. doi:10.1021/jf502950u

Lizko N (1991) Problems of microbial ecology in man space mission. Acta Astronaut 23:163–169. doi:10.1016/0094-5765(91)90115-L

Lynch S, Brodie E, Matin A (2004) Role and regulation of sigma S in general resistance conferred by low-shear simulated microgravity in *Escherichia coli*. J Bacteriol 186:8207–8212. doi:10.1128/JB.186.24.8207-8212.2004

Lynch S, Mukundakrishnan K, Benoit M, Ayyaswamy P, Matin A (2006) *Escherichia coli* Biofilms formed under low shear modelled microgravity in ground-based system. Appl Environ Microb 72:7701–7710. doi:10.1128/AEM.01294-06

Mauclaire L, Egli M (2010) Effect of simulated microgravityon growth and production of exopolymeric substances of *Micrococcus luteus* space and earth isolates. FEMS Immunol Med Microbiol 59:350–356. doi:10.1111/j.1574-695X.2010.00683.x

McLean RJC, Cassanto JM, Barnes MB, Koo JH (2001) Bacterial biofilm formation under microgravity conditions. FEMS Microbiol Lett 195:115–119. doi:10.1111/j.1574-6968.2001.tb10507.x

Meier PJ, Stieger B (2002) Bile salt transporters. Annu Rev Physiol 64: 635–661. doi:10.1146/annurev.physiol.64.082201.100300

Mermel LA (2013) Infection prevention and control during prolonged human space travel. Healthcare. Epidemiology 56:123–130. doi:10.1093/cid/cis861

Min BR, Pinchak WE, Merkel R, Walker S, Tomita G, Anderson RC (2008) Comparative antimicrobial activity of tannin extracts from perennial plants on mastitis pathogens. Sci Res Essays 3:66–73

Nickerson C, Ott C, Mister S, Morrow B, Burns-Keliher L, Pierson D (2000) Microgravity as a novel environmental signal affecting *Salmonella enterica Serovar Typhimurium* virulence. Infect Immun 68:3147–3152. doi:10.1128/IAI.68.6.3147-3152.2000

Ouwehand AC, Isolauri E, Kirjavainen PV, Salminen SJ (1999) Adhesion of four *Bifidobacterium* strains to human intestinal mucus from subjects in different age groups. FEMS Microbiol Lett 172:61–64. doi:10.1111/j.1574-6968.1999.tb13450.x

Rosenzweig J, Abogunde O, Thomas K, Lawal A, Nguyen Y, Sodipe A, Jejelowo O (2010) Spaceflight and modeled microgravity effects on microbial growth and virulence. App Microbiol Biot 85:885–891. doi:10.1007/s00253-009-2237-8

Rosado H, Doyle M, Hinds J, Taylor PW (2010) Low-shear modelled microgravity alters expression of virulence determinants of *Staphylococcus aureus*. Acta Astronaut 66:408–413. doi:10.1016/j.actaastro.2009.06.007

Shao D, Bartley GE, Yokoyama W, Pan Z, Zhang H, Zhang A (2013) Plasma and hepatic cholesterol-lowering effects of tomato pomace, tomato seed oil and defatted tomato seed in hamsters fed with high-fat diets. Food Chem 139:589–596. doi:10.1016/j.foodchem.2013.01.043

Shi F, Wang YC, Zhao TZ, Zhang S, TY D, Yang CB, Li YH, Sun XQ (2012) Effects of simulated microgravity on human umbilical vein endothelial cell angiogenesis and role of the PI3K-Akt-eNOS signal pathway. PLoS One 7:e40365. doi:10.1371/journal.pone.0040365

Soccol C, Vandenberghe L, Spier M, Medeiros A, Yamaguishi C, Lindner J (2010) The potential of probiotics: a review. Food Technol. Biotech 48:413–434

Taylor PW, Sommer AP (2005) Towards rational treatment of bacterial infections during extended space travel. Int J Antimicrob Ag 26: 183–187. doi:10.1016/j.ijantimicag.2005.06.002

Vesterlund S (2009) Mechanisms of probiotics: production of antimicrobial substances. In: Lee YK, Salminen S (eds) Handbook of probiotics and prebiotics, 2nd edn. John Wiley & Sons, Inc., Hoboken

Vukanti R, Model MA, Leff LG (2012) Effect of modeled reduced gravity conditions on bacterial morphology and physiology. BMC Microbiol 12:1–11. doi:10.1186/1471-2180-12-4

Vukanti R, Mintz E, Leff L (2008) Changes in gene expression of *E. coli* under conditions of modeled reduced gravity. Microgravity Sci Tec 20(1):41–57. doi:10.1007/s12217-008-9012-9

Wang H, Yang YJ, Qian HY, Zhang Q, Xu H, Li JJ (2012) Resveratrol in cardiovascular disease: what is known from current research? Heart Fail Rev 17:437–448. doi:10.1007/s10741-011-9260-4

Yin S, Zhai Z, Wang G, Na H, Luo Y, Hao Y (2011) A novel vector for lactic acid bacteria that uses a bile salt hydrolase gene as a potential food-grade selection marker. J Biotechnol 152:49–53. doi:10.1016/j.jbiotec.2011.01.018