

# Effects of simulated microgravity and spaceflight on morphological differentiation and secondary metabolism of *Streptomyces coelicolor* A3(2)

Bing Huang · Ning Liu · Xiaoying Rong · Jisheng Ruan · Ying Huang

Received: 22 October 2014 / Revised: 31 December 2014 / Accepted: 5 January 2015 / Published online: 30 January 2015  
© Springer-Verlag Berlin Heidelberg 2015

**Abstract** As well-known antibiotic-producing and filamentous bacteria, streptomycetes can be an ideal model to study the effects of microgravity on microbial development and antibiotic production. In this study, the model organism *Streptomyces coelicolor* A3(2) was exposed to simulated microgravity (SMG) on a rotating clinostat and microgravity ( $\mu\text{g}$ ) on the Shenzhou-8 spacecraft. The strain exhibited some similar responses under both conditions. Compared with the controls, its life cycle in agar medium was shortened relatively, and the sporulation process was accelerated with higher accumulation of the gray spore pigment; the liquid cultures yielded more cell biomass, coupled with thicker, more fragmented, and well-dispersed hyphae of the  $\mu\text{g}$  spaceflight samples. Global transcriptional analysis verified that most of the differentially expressed genes involved in morphological differentiation of *S. coelicolor* were upregulated during days 4–6 under SMG conditions, notably the *whi* genes (*whiD*, *sigF*, and *whiE*). Production of actinorhodin (ACT) in agar cultures decreased under both conditions while undecylprodigiosin (RED) was produced earlier, which were consistent with the transcriptional levels of *act* and *red* gene clusters. Meanwhile, expression of the gene clusters for calcium-dependent antibiotic (CDA), methylenomycin (MMY), and a cryptic polyketide (CPK) was unchanged,

downregulated, and upregulated, respectively, the latter of which might contribute to the enhanced activity of *S. coelicolor* against *Bacillus subtilis* under microgravity. Our study provides new insights into the morphological and secondary metabolic responses of streptomycetes to microgravity.

**Keywords** *Streptomyces coelicolor* · Spaceflight · Simulated microgravity · Morphological differentiation · Secondary metabolism · Shenzhou-8 space mission

## Introduction

Microbes have evolved for some 3.8 billion years and can survive in many extreme environments including space, but how they respond and adapt themselves to these environments, especially microgravity in space, remains unclear. Spaceflight and ground-simulated microgravity (SMG) experiments have suggested that microgravity can affect cellular processes and functions in microorganisms, such as cell growth (Kacena et al. 1999; Lawal et al. 2013) and differentiation (Mennigmann and Lange 1986; Van Mulders et al. 2011), biofilm formation (Crabbé et al. 2008; Lynch et al. 2006), gene expression (Crabbé et al. 2011; Wilson et al. 2007; Wilson et al. 2002b), secondary metabolism (Demain and Fang 2001; Lam et al. 1998), cell virulence (Crabbé et al. 2010; Lawal et al. 2010), and cell resistance (Lynch et al. 2004; Wilson et al. 2002a). Although the effects of microgravity on microbial growth and metabolism have been studied for more than 50 years, systematic and comprehensive studies of the genetic and phenotypic responses of microorganisms to microgravity environment in space are still insufficient due to technological and logistical hurdles. Data of microbial growth and metabolism in space were often based on small

**Electronic supplementary material** The online version of this article (doi:10.1007/s00253-015-6386-7) contains supplementary material, which is available to authorized users.

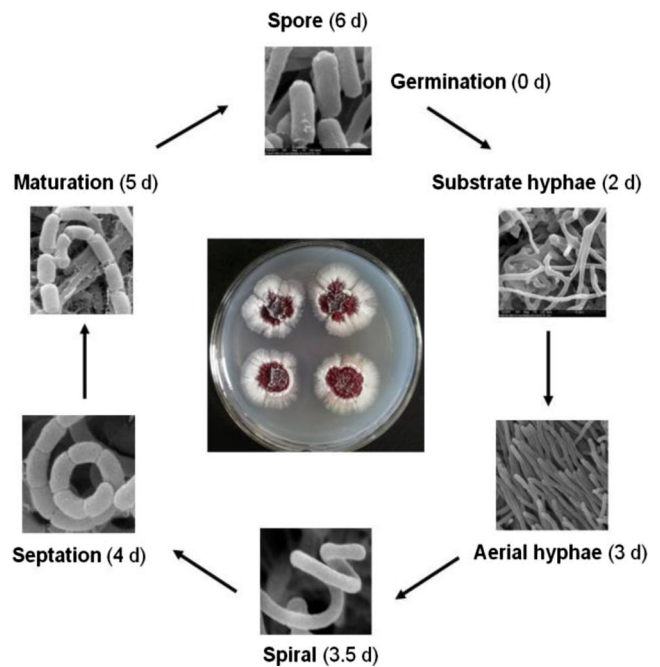
B. Huang · N. Liu · X. Rong · J. Ruan · Y. Huang (✉)  
State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, NO. 1 Beichen West Road, Chaoyang District Beijing 100101, China  
e-mail: huangy@im.ac.cn

B. Huang  
University of Chinese Academy of Sciences, Beijing 100049, China

samples and lack of proper control groups (Herranz et al. 2013), thus leading to compromised experimental results. Meanwhile, the selected strains, cultivation patterns, and methods of microgravity varied according to purposes of research, and conflicting results were often reported in different experiments (Benoit and Klaus 2007). Previous spaceflight experiments have shown that suspension cultures of *Escherichia coli* and *Bacillus subtilis* exhibited increased cell growth in the spaceflight environment (Kacena et al. 1999; Klaus et al. 1997; Mennigmann and Lange 1986). However, some other studies showed that the *E. coli* and *B. subtilis* grown on solid agar on Space Shuttle Mission STS-63 did not experience an increased final cell mass, and changes in other growth characteristics might occur when grown under various gravitational conditions (Kacena and Todd 1997; Kacena et al. 1997).

At present, long-term real microgravity can be achieved only in spaceflight. Recently, taking advantage of the cooperation between China and Germany in the scientific utilization of China Manned Space Program, 17 biological experiments were accomplished in the German SIMBOX (Science in Microgravity Box) onboard the Chinese spacecraft Shenzhou-8 (Preu and Braun 2014), and a number of new findings have been obtained (Nasir et al. 2014; Paulsen et al. 2014; Pietsch et al. 2013). Shenzhou-8 was launched atop a Long March-2 F carrier rocket at the Jiuquan Satellite Launch Center (Jiuquan, China) on November 1, 2011 and landed after a 16.5-day mission on the main landing site at the Gebi desert in Inner Mongolia. Because of the rarity and costliness of microgravity experiments in space, several ground-based facilities (GBFs) with different physical concepts have been developed to simulate microgravity effects on the ground (Herranz et al. 2013). Clinostats, for instance, can generate along with rotation a continuous change of the direction of the gravity vector on the sample, resulting in the gravity vector being time-averaged to near zero (Klaus 2001). The two-dimensional (2D) clinostat, a classical and well-established paradigm, has been widely used till today to study the effects of microgravity on biological samples. Moreover, several studies have shown that results from various model systems using 2D clinorotation are similar to those found under real microgravity conditions (Brungs et al. 2011; Eiermann et al. 2013; Hemmersbach et al. 2006; Thiel et al. 2012).

Streptomycetes are high genome G+C, gram-positive, filamentous bacteria that produce a variety of bioactive natural products and have a complex life cycle, undergoing development and differentiation from spores to substrate hyphae, aerial hyphae, spore chains, and mature spores (Fig. 1) (Bentley et al. 2002; Flardh and Buttner 2009). Meanwhile, morphological differentiation and secondary metabolism of streptomycetes are generally sensitive to extracellular environmental signals and stresses, including nutrients, heat, osmotic pressure, and so on (Bibb 2005; Viollier et al. 2003). Therefore,



**Fig. 1** The morphological features in main stages of *Streptomyces coelicolor* A3(2) life cycle (grown on yeast-starch agar) observed by SEM

streptomycetes can be an ideal model to study the microbial responses in morphology and secondary metabolism to microgravity environment. However, effects of microgravity on morphological development and differentiation of streptomycetes have been little studied and described. Regarding the secondary metabolism, previous studies demonstrated that the production of  $\beta$ -lactam antibiotics by *Streptomyces clavuligerus* and rapamycin by *Streptomyces hygroscopicus* was inhibited under SMG using NASA rotating-wall bioreactors (RWBs) (Fang et al. 1997; Fang et al. 2000), and the productivity of actinomycin D by *Streptomyces plicatus* was increased during the US Space Shuttle Mission STS-80 (Lam et al. 2002), corroborated by later findings for the early sample points (days 8 and 12) aboard the International Space Station (Benoit et al. 2006). Collectively, these studies suggest that microgravity environment could alter secondary metabolism in streptomycetes. Nevertheless, molecular evidence for the metabolic phenotypes has never been reported.

The model strain *Streptomyces coelicolor* A3(2) is a representative soil-dwelling, antibiotic-producing streptomycete, with its genome containing more than 20 secondary metabolite clusters and 965 genes encoding proteins predicted to have a regulatory role (Bentley et al. 2002). In this study, we investigated the growth, morphological and secondary metabolic responses of *S. coelicolor* A3(2) exposed to ground SMG by clinostat rotation and real microgravity environment by Shenzhou-8 space mission, respectively. We observed interesting similarities in acceleration of morphological differentiation, increase of bacteriostatic activity, and variations of

secondary metabolite production in both SMG and space microgravity environments. The phenotypic changes were further correlated to the genetic responses at transcriptional level by microarray gene expression profiling and qRT-PCR analysis.

## Materials and methods

### Strains and growth conditions

*S. coelicolor* A3(2) (=DSM 40783=JCM 4979), *S. coelicolor* M145 (=ATCC BAA-471), and *B. subtilis* CGMCC 1.2428 were revived on yeast-starch agar (yeast extract, 2.0 g/L; soluble starch, 10.0 g/L; agar, 15.0 g/L) at 28 °C and maintained as suspensions of spores (streptomycetes,  $10^8$  spores/mL) or cells (bacillus,  $10^6$  cells/mL) in 20 % (v/v) glycerol at –20 °C. The strains were cultured in experiment unique equipment (EUE, Astrium EADS, Germany; <http://www.astrium-na.com/2012AstriumSpaceBiologyProductCatalog.pdf>) each with eight cell culture chambers, four on one side for solid culture and four on the other side for liquid culture, sealed with gas permeable biofoil (Astrum EADS, Germany) (Fig. S1; Electronic supplementary material). The streptomycetes were grown as pure cultures and cocultures with *B. subtilis*, and colonies were grown by spotting 5- $\mu$ L spore suspensions each spot onto yeast-starch agar (3 mL per chamber of the EUE). For solid coculture, 10- $\mu$ L cell suspensions of *B. subtilis* were well mixed into 50-mL yeast-starch agar medium before pouring into the chamber. For liquid culture, 100- $\mu$ L streptomycete spore suspensions were inoculated into 3-mL yeast-starch liquid medium in the chamber, followed by inoculating 5- $\mu$ L *B. subtilis* cell suspensions for coculture. The strains were also cultured on agar plates (3.0 cm of radius and 1 cm of thickness, containing 15-mL yeast-starch agar medium, sealed with parafilm) and in centrifuge tubes (50 mL, containing 30-mL yeast-starch liquid medium) for clinostat experiments.

### Clinostat-SMG experiment

A 2D clinostat with a horizontal axis and a radius of 15.0 cm, manufactured by the National Space Science Center, Chinese Academy of Sciences, was used in this study to simulate microgravity effects on the ground (Fig. S2). The EUEs, plates, and centrifuge tubes containing test group samples were fixed to the clinostat and rotated at 10.0 rpm and an average rotation radius of 10.0 cm. This created a residual gravity level of  $(7.8–14.5) \times 10^{-3}$  g according to the reference method (van Loon 2007). The control groups were grown under the static 1-g condition, and all other conditions were the same. The temperature for the SMG experiment was 28 °C. The samples were collected every 24 h by scraping the surface mycelium

of agar cultures or centrifuging the cells of liquid cultures for further analyses.

### Shenzhou-8 spaceflight experiment

The strains were incubated in SIMBOX (Astrum EADS, Germany) during the Shenzhou-8 space mission ( $\mu$ g =  $10^{-3}$ – $10^{-4}$  g). SIMBOX is an advanced space incubator with 42 separate slots for experimental containers and a 1-g centrifuge to simulate gravity in space (Prasad et al. 2004; Preu and Braun 2014). The microbial samples were inoculated into the EUE about 12 h before the launch of Shenzhou-8, and two EUEs were loaded into the SIMBOX about 10 h before launch, one in a static slot ( $\mu$ g position,  $\mu$ g) and one in a centrifuge slot (simulated 1-g position, S-1 g) (Fig. S3). The centrifuge was started after launch, and the temperature of SIMBOX throughout the mission was maintained at  $23 \pm 0.5$  °C. The Shenzhou-8 spacecraft reentered and landed after 16.5 days of spaceflight, and the microbial samples in SIMBOX were collected 6 h after landing at Payload Integration Test Center (PITC) of General Establishment of Space Science and Application (GESSA), Chinese Academy of Sciences (Beijing, China).

The corresponding ground controls of the experiment were conducted 1 day later at the PITC according to the spaceflight scenario. And, the parallel clinostat-SMG experiment was executed at our laboratory in Beijing following the same procedure except for temperature.

### Cultural and morphological characterization

The cultural and morphological properties of streptomycetes were examined both by the eye and microscopically following 2-, 4-, 6-, and 16.5-day clinostat-SMG experiment and recovery of the spaceflight samples. For scanning electron microscopy (SEM), the samples were fixed in 2.5 % glutaraldehyde (in 0.1 M phosphate buffer, pH 7.0), dehydrated in 50, 70, 85, 95, and 100 % methanol successively, subjected to CO<sub>2</sub> critical point drying and sputter-coated with gold, and then observed using a QUANTA 200 (30 kV) SEM (FEI). To measure the growth rate of *S. coelicolor* A3(2), triplicate samples of mycelium were washed twice with distilled water and collected on a preweighed filter by vacuum filtration before weighing (fresh cell weight). The filters with the mycelium were reweighed after freeze-drying at –80 °C for 24 h (dry cell weight).

### Bacteriostatic activity assay

Bacteriostatic activity of *S. coelicolor* A3(2) under simulated and space microgravity conditions was assayed by calculating colony-forming units (CFUs/mL) of the cocultured indicator

strain *B. subtilis* CGMCC 1.2428. For the agar culture, half of the 3-mL agar block was cut uniformly into 32 small pieces, and *B. subtilis* CFUs in each piece were counted directly under a microscope; for the liquid culture, the surviving *B. subtilis* CFUs were quantified by serial dilution plating. CFUs of pure cultures of *B. subtilis* were also calculated to determine the effect of microgravity on the growth of the indicator.

#### Chemical analysis

The spore pigment (TW95a, a product of the type II PKS gene cluster named *whiE*) of *S. coelicolor* A3(2) grown on yeast-starch agar medium was extracted from the aerial spore mass and measured by OD<sub>404</sub> according to its characteristic UV absorption spectrum (Yu et al. 1998). A strong correlation ( $r^2=0.9991$ ) was found between the concentration of the spore pigment and OD<sub>404</sub> (Fig. S4). Undecylprodigiosin (RED) and actinorhodin (ACT) were extracted from agar cultures and quantified spectrophotometrically as previously described (Gao et al. 2012; Kang et al. 1998).

#### RNA extraction, labeling, and microarray analysis

The *S. coelicolor* A3(2) DNA microarrays were custom designed using the Agilent eArray 5.0 program according to the manufacturer's recommendations (<http://earray.chem.agilent.com/earray/>). The genechip specification was 8\*15 K (Agilent). The genome sequence was downloaded from [http://www.ncbi.nlm.nih.gov/genome?Db=genome&Cmd=Search&Term=NC\\_003888, NC\\_003903.1, NC\\_003904.1](http://www.ncbi.nlm.nih.gov/genome?Db=genome&Cmd=Search&Term=NC_003888,NC_003903.1,NC_003904.1). Each gene in the chromosome and two plasmids was represented by one 60-nt oligonucleotide probe. Out of the total 8116 genes, 680 genes associated with morphological differentiation and secondary metabolism were replicated nine times each. Total RNA of *S. coelicolor* A3(2) was extracted from the 4- and 6-day samples of SMG experiment and the 16.5-day samples of spaceflight experiment by using TRIZOL (Invitrogen) and purified using the RNeasy Mini Kit (Qiagen). The quality and quantity of RNA were examined by 1 % agarose gel electrophoresis (Agilent Bioanalyzer 2100) and with a UV spectrophotometer (NanoDrop ND-1000, USA). Two micrograms of RNA was reverse-transcribed into cDNA and then transcribed into cRNA using the Low RNA Input Linear Amplification Kit (Agilent). After purification, 4 µg of cRNA was labeled with Cy3 NHS ester (GE Healthcare) and further purified. The labeled cRNAs were hybridized to the Agilent GeneChip arrays using the Gene Expression Hybridization Kit (Agilent) at 65 °C for 17 h with a rotation at 10 rpm. Arrays were washed twice using the Gene Expression Wash Buffer Kit (Agilent), and then scanned with an Agilent Microarray Scanner System

(G2565BA, Agilent) at a resolution of 5 µm, dual pass at 100 and 10 % PMT settings. The two data sets were combined automatically, and signal intensities were normalized using the Feature Extraction Software (Agilent). Data were analyzed using GeneSpring Software 5.0 (Agilent). Microarray analyses were performed on three (SMG experiment) or one (spaceflight experiment) biological replicates. Differentially expressed genes were selected with  $P<0.05$  and fold change (FC)  $\geq 2.0$  in a *t* test. Pathways were analyzed by the SAS pathway enrichment suite (Shanghai Biotechnology Corporation, Shanghai, China) using the genes with an FC of  $\geq 2.0$ . Genes with an FC of  $\geq 1.5$  but less than 2.0 were analyzed when needed. Microarray data obtained in this study have been deposited in the Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo/>) under accession numbers GSE53748, GSE53749, and GSE53750.

#### Real-time qRT-PCR analysis

The qRT-PCR primers used in this study are listed in Table S1 (Electronic supplementary material). First-strand cDNA synthesis was carried out with the Superscript III first-strand Synthesis System (Invitrogen) using 500 ng total RNA following the manufacturer's instructions. All cDNA synthesis reactions included a replicate reaction without reverse transcriptase to ensure the complete removal of contaminating DNA from the RNA samples. The PCR was done with SYBR®Premix Ex Taq™ (Takara, Dalian, China) in a reaction mixture containing 8.8 µL cDNA, 10 µL SYBR®Premix Ex Taq™ (2×), 0.4 µL ROX Reference Dye (50×), and 0.2 µM of each pair of primers. Thermal cycling conditions were 50 °C for 2 min and 95 °C for 10 min, followed by 40 three-step amplification cycles consisting of denaturation at 95 °C for 10 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. A final dissociation stage was run to generate a melting curve and consequently verify the specificity of the amplification products. PCRs were carried out in triplicate, and each experiment was repeated three times, using an ABI Prism 7000 sequence detection system (Applied Biosystems, CA, USA). The transcriptional levels of target genes were normalized internally to the level of *hrdB* gene.

#### Statistical analysis

Data collected in this study were evaluated by the Student's *t* test method. Unless otherwise stated, the statistical analysis of Microsoft Excel 2010 for Windows was used. Probability values of  $P<0.05$  were considered to be significant. A gene's expression was considered to be significantly altered if the transcript number changed  $\geq 2$ -fold with  $P<0.05$  in a Student's *t* test.

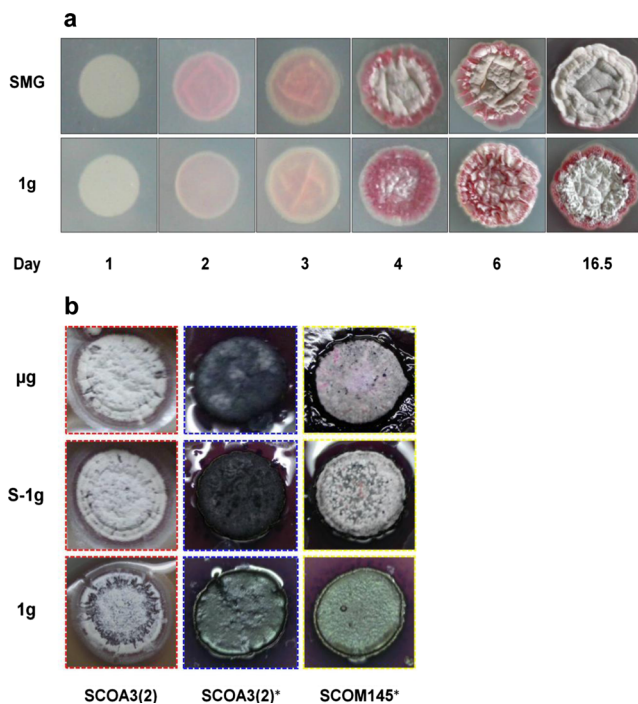


## Results

### Effects of SMG and spaceflight on morphological differentiation and growth of *S. coelicolor*

It was obvious from the cultural characteristics of *S. coelicolor* A3(2) that the colony color of SMG samples turned to red and then gray earlier than that of the 1-g controls, with higher accumulation of the gray spore pigment on colonies from day 4 (Fig. 2a). The spaceflight samples were examined after landing and recovery. Again, the 16.5-day  $\mu\text{g}$  agar cultures of *S. coelicolor* A3(2) also accumulated relatively more gray spore pigment on colony surfaces than the S-1 g and 1-g controls (Fig. 2b). Similar results were found for the  $\mu\text{g}$  spaceflight sample of *S. coelicolor* M145 as well, which developed even more abundant aerial hyphae than the two controls (Fig. 2b).

Micrographs revealed that, under SMG conditions, *S. coelicolor* A3(2) on agar medium produced aerial hyphae earlier, which spiraled and fragmented into spores ahead of the 1-g controls, and the amount of mature spores characteristic of



**Fig. 2** Colony features of *S. coelicolor* cultured on yeast-starch agar under SMG and spaceflight conditions compared to the controls. **a** Colony features under SMG after 1, 2, 3 (reverse side), 4, 6 and 16.5 days (front side). **b** Colony features after the 16.5-day spaceflight experiment. *SCOA3(2)* framed in red indicates *S. coelicolor* A3(2) cultured purely; *SCOA3(2)\** framed in blue indicates *S. coelicolor* A3(2) cocultured with the indicator strain *B. subtilis*; *SCOM145\** framed in yellow indicates *S. coelicolor* M145 cocultured with *B. subtilis*. 1g static 1-g conditions on the ground;  $\mu\text{g}$  the  $\mu\text{g}$  position in a static slot of the SIMBOX on Shenzhou-8; S-1 g the simulated 1-g position in a centrifuge slot of the SIMBOX on Shenzhou-8

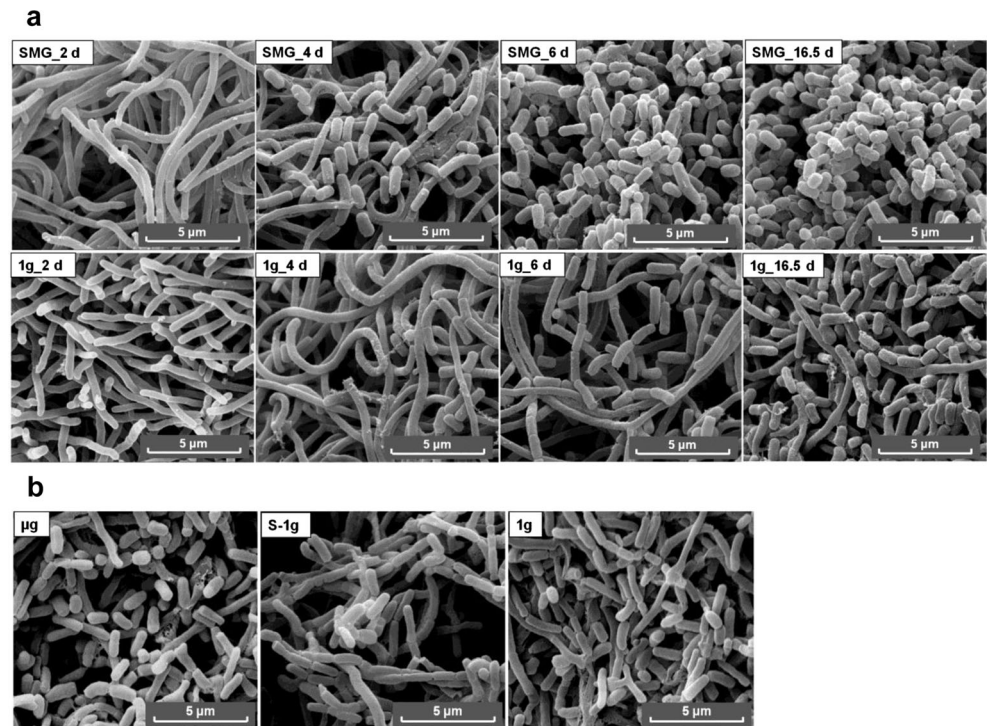
short- and blunt-rod shape was obviously higher than that of the controls after 6-day growth (Fig. 3a). However, the morphological feature of mycelium in SMG liquid cultures was similar to the controls, with mycelium settled to the base of the growth chamber and aggregated into clusters (data not shown). Micrographs of the 16.5-day spaceflight samples also showed more mature spores of agar cultures in the  $\mu\text{g}$  group than in the S-1 g and 1-g control groups that still contained some long rod-shaped spores (Fig. 3b). Moreover, mycelium in the  $\mu\text{g}$  liquid cultures showed high uniformity and dispersity, while mycelium in the two controls aggregated into large and dense clusters (Fig. 4a); and the hyphae in the  $\mu\text{g}$  liquid cultures were thick and fragmented obviously into segments compared to the two controls (Fig. 4b).

The effect of microgravity on growth of *S. coelicolor* was also investigated by measuring the cell weight. As shown in Fig. 5, similar growth curves were observed for the agar cultures of SMG and 1-g control groups, but in liquid cultures, cells under SMG conditions grew more rapidly than the 1-g control cells. The recovered 16.5-day  $\mu\text{g}$  spaceflight samples showed in colony diameter no obvious difference from the S-1 g spaceflight and 1-g ground control groups, while the liquid cultures of  $\mu\text{g}$  samples accumulated more cell mass ( $158.73 \pm 0.55$  mg/chamber, mean  $\pm$  root mean square error [RMSE], measured by fresh cell weight) than the S-1 g ( $133.50 \pm 0.51$ ) and 1-g ( $124.80 \pm 0.74$ ) controls.

### Effects of SMG and spaceflight on bacteriostatic activity and secondary metabolite production

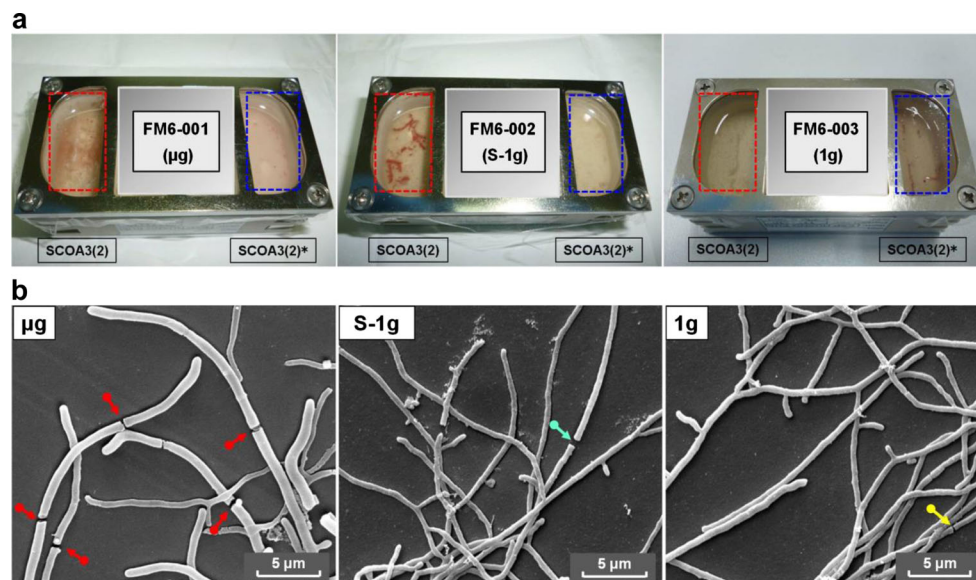
*S. coelicolor* A3(2) has been reported to produce at least five antibiotics: actinorhodin (ACT), undecylprodigiosins (RED), calcium-dependent ionophore antibiotic (CDA), methylenomycin (MMY), and cryptic polyketide (CPK) (Liu et al. 2013), of which ACT (Wright and Hopwood 1976), CDA (Lakey et al. 1983), CPK (Gottelt et al. 2010), and MMY (Haneishi et al. 1974) exhibited bacteriostatic activity. To determine the overall bacteriostatic activity under SMG and spaceflight, *B. subtilis* was used as an indicator and cocultured with *S. coelicolor* A3(2). According to statistical analysis of *B. subtilis* CFUs, the final number (CFUs/mL, mean  $\pm$  standard deviation [SD]) of *B. subtilis* viable cells in agar and liquid samples under SMG conditions were  $(1.637 \pm 0.022) \times 10^3$  and  $(2.907 \pm 0.018) \times 10^4$ , respectively, while those in the 1-g control groups were  $(3.044 \pm 0.028) \times 10^3$  and  $(9.367 \pm 0.088) \times 10^4$ , respectively, which were significantly higher ( $P < 0.05$ ). On the contrary, the pure culture of *B. subtilis* exhibited increased cell growth under SMG compared to the 1-g control, particularly in the liquid medium (Fig. S5). The 16.5-day  $\mu\text{g}$  spaceflight samples contained *B. subtilis* CFUs/mL of  $(8.070 \pm 0.230) \times 10^2$  and  $(8.978 \pm 0.222) \times 10^3$  in agar and liquid chambers, respectively, while the S-1 g controls yielded  $(1.757 \pm 0.018) \times 10^3$  and  $(2.486 \pm$

**Fig. 3** Microscopic morphological features of *S.coelicolor* A3(2) grown on yeast-starch agar under SMG and spaceflight conditions compared to the controls. **a** SEM micrographs after growth under SMG and 1-g conditions for 2, 4, 6, and 16.5 days. **b** SEM micrographs after the 16.5-day spaceflight experiment. *Ig* static 1-g conditions on the ground;  $\mu g$  the  $\mu g$  position in a static slot of the SIMBOX on Shenzhou-8; *S-1g* the simulated 1-g position in a centrifuge slot of the SIMBOX on Shenzhou-8



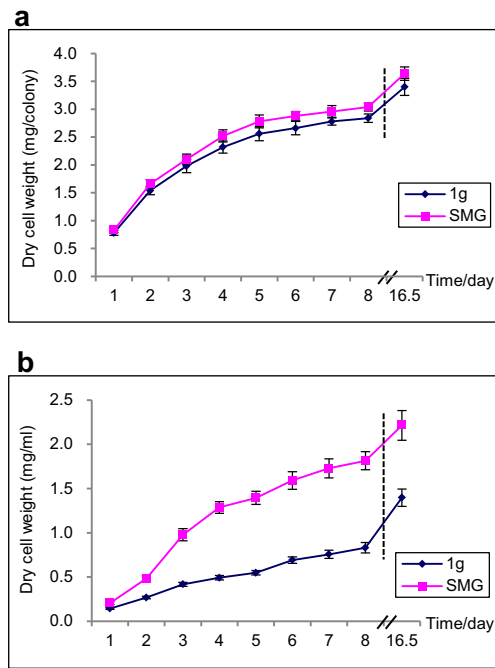
$0.034) \times 10^4$ , and the 1-g controls yielded  $(2.539 \pm 0.027) \times 10^3$  and  $(7.022 \pm 0.051) \times 10^4$ , respectively, which were also significantly higher ( $P < 0.05$ ). These results indicated that *S. coelicolor* exhibited stronger bacteriostatic activity against *B. subtilis* under microgravity conditions.

In accordance with the observed morphological characteristics, measuring the gray spore pigment TW95a (a product of the *whiE* gene cluster) of colonies at  $OD_{404}$  quantitatively revealed that the spore pigment was accumulated faster and more under SMG conditions than under 1 g (Fig. 6a). The blue



**Fig. 4** Cultural and morphological features of *S. coelicolor* A3(2) grown in yeast-starch liquid medium after the 16.5-day spaceflight experiment. **a** Cultural features in the EUEs. *SCOA3(2)* framed in red indicates *S. coelicolor* A3(2) cultured purely; *SCOA3(2)\** framed in blue indicates *S. coelicolor* A3(2) cocultured with *B. subtilis*. FM6-001, FM6-002, and FM6-003 are the serial numbers of EUEs in the mission.

**b** SEM micrographs of *S. coelicolor* A3(2) mycelium. Arrows indicate fragmentations of the mycelium. *Ig* static 1-g conditions on the ground;  $\mu g$  the  $\mu g$  position in a static slot of the SIMBOX on Shenzhou-8; *S-1g* the simulated 1-g position in a centrifuge slot of the SIMBOX on Shenzhou-8

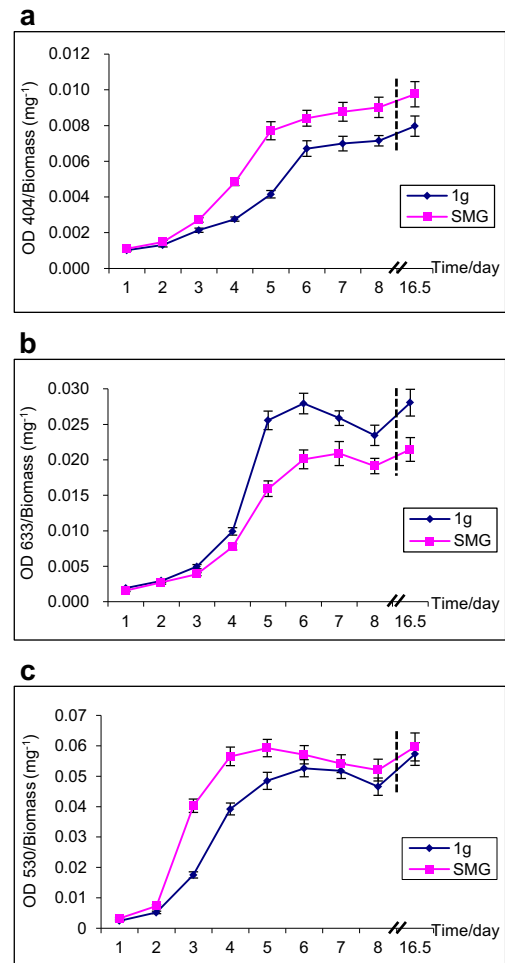


**Fig. 5** Growth curves of *S. coelicolor* A3(2) cultured in yeast-starch agar (a) and liquid (b) media under SMG as compared to the 1-g controls. The growth curves were measured by dry cell weight after growing for 1–8 and 16.5 days

aromatic polyketide antibiotic ACT and the red oligopyrrole prodiginine antibiotic RED, two well-known secondary metabolites of *S. coelicolor*, were also measured for the agar cultures. The production of ACT (OD<sub>633</sub>/mg biomass) was reduced with less final accumulation under SMG conditions (Fig. 6b), while RED (OD<sub>530</sub>/mg biomass) was produced earlier with nearly the same final accumulation (Fig. 6c). In the spaceflight experiment, both the ACT and RED productions (OD/chamber, mean±RMSE) in the recovered μg samples (0.316±0.002; 0.505±0.001) were relatively less than those in the S-1 g spaceflight (0.372±0.002; 0.534±0.003) and 1-g ground control (0.453±0.003; 0.591±0.002) groups.

#### Transcriptome variation under SMG and spaceflight conditions

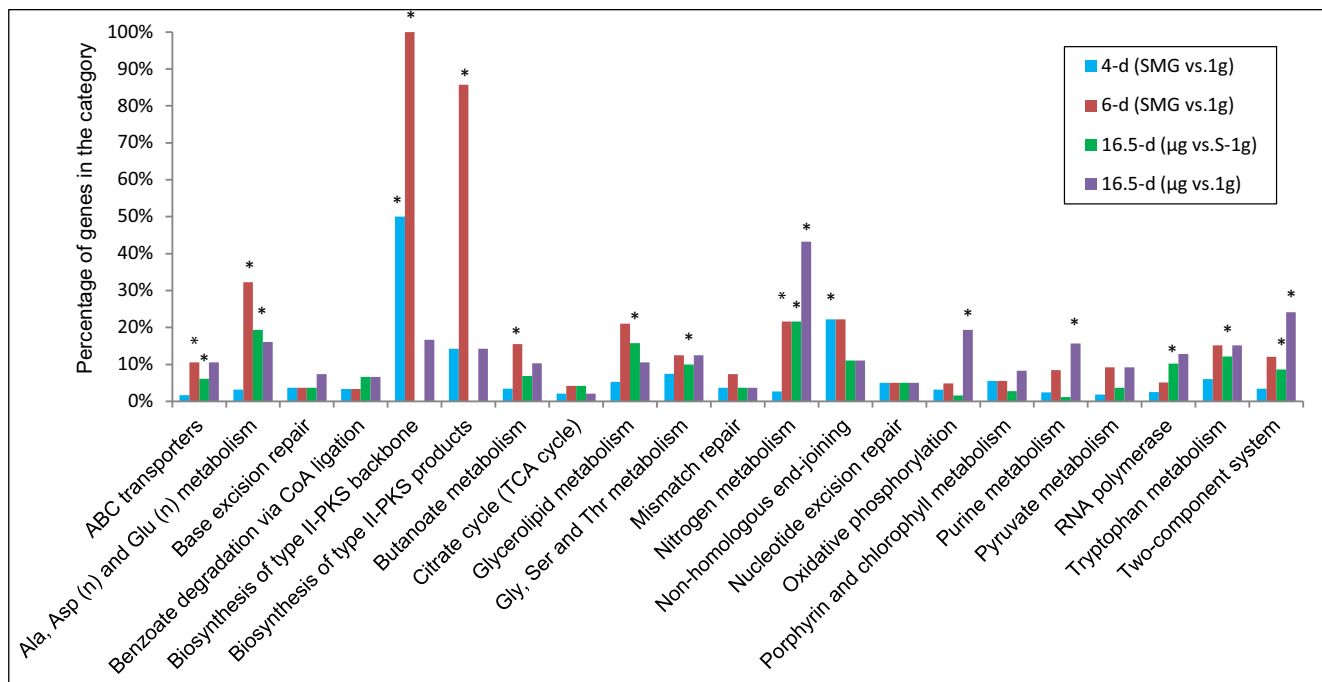
Microarray analysis revealed a significant fraction of gene expression variation (2.4–7.0 %, 2.0-fold threshold,  $P < 0.05$ ) across the transcriptome under SMG and spaceflight conditions, as compared to the 1-g and S-1 g controls. At day 4 under SMG conditions, 196 out of the total 8116 genes were significantly differentially expressed ( $\geq 2.0$ -fold,  $P < 0.05$ ), with 159 genes upregulated and 37 genes downregulated, which were enriched in 26 out of the total 122 pathways. The differentially expressed genes increased to 552 at day 6 under SMG conditions, with 362 genes upregulated and 190 genes downregulated, enriched in 52 pathways. The 16.5-day spaceflight samples showed 271 and 570 genes significantly



**Fig. 6** Secondary metabolite analysis of *S. coelicolor* A3(2) cultured on yeast-starch agar under SMG and 1-g conditions. The productions of secondary metabolites were quantified spectrophotometrically after growing for 1–8 and 16.5 days. a The gray spore pigment TW95a measured by OD<sub>404</sub>. b ACT measured by OD<sub>633</sub>. c RED measured by OD<sub>530</sub>

differentially expressed compared to the control samples of S-1 g and 1 g, respectively, enriched in 48 and 64 pathways, respectively. Nineteen pathways were enriched in all four experimental cases, however, with no more than 50 % of genes in the category (Fig. 7). Besides, it is remarkable that the two pathways responsible for biosynthesis of type II polyketides (KEGG Pathway IDs: sco01056 and sco01057) were both significantly enriched under SMG conditions, with most of the genes (>85 %) differentially expressed at day 6 and 50 % genes for biosynthesis of the backbone altered 2 days earlier (Fig. 7). These results indicated that the biosynthesis of spore pigment TW95a and antibiotic ACT, the only two type II polyketides produced by *S. coelicolor*, were likely to be altered during cultivation under microgravity conditions, which is in line with the phenotypic results. Based on the whole genome transcriptional data and phenotypic data, we





**Fig. 7** Comparative analyses of the enriched pathways based on microarray data. Pathways were analyzed by the SAS pathway enrichment suite (Shanghai Biotechnology Corporation, Shanghai, China) using the genes with an FC of  $\geq 2.0$ . Nineteen pathways were

enriched in all four experimental cases, and two pathways responsible for biosynthesis of type II polyketides were enriched in three cases, significantly under SMG conditions. “\*” indicates enrichment test  $P$  value  $< 0.05$

focused on the genes involved in morphological differentiation and secondary metabolism in *S. coelicolor* A3(2) for further analysis.

#### Effects of SMG and spaceflight on genes involved in morphological differentiation

A series of the differentially expressed involved in morphological differentiation and development of streptomycetes, such as *chp*, *dev*, *ku-lig*, *pbp*, *rdd*, *sig*, *ssg*, sortase gene, *wbl*, *whi*, and so on (Table 1 and Table S2), mainly associated with aerial hyphae erection, sporulation, spore germination, cell wall structure, spore structure, and development-associated secondary sigma factors (Kirby et al. 2011). Among these genes, 31 were significantly upregulated and three downregulated at day 4 in SMG, and these numbers changed to 23 and eight at day 6 in SMG. It is noticeable from Table 1 that the transcription of most *whi* genes (16 in total) were upregulated at days 4 and 6 in SMG, notably the late-stage *whiE* cluster of genes (SCO5314–SCO5320, *whiEI*–*whiEVII* except for *whiEVIII*) coding for biosynthesis of the type II polyketide spore pigment, and another two late-stage *whi* genes (*whiD* and *sigF*) that regulate positively the *whiE* cluster. The six early-to-mid *whi* genes (*whiG*, *whiA*, *whiB*, *whiJ*, *whiH*, *whiI*) were not significantly altered. Most of the other differentially expressed genes involved in the development of *S. coelicolor* A3(2) were also upregulated during days 4–6

under SMG conditions, notably those encoding secondary RNA polymerase sigma factors (*sigB*, *sigL*, and *sigM*) (Table S2).

As a further test, we used qRT-PCR to examine the transcription profiles of select genes at days 4 and 6 in SMG, including *whiD*, *sigF*, *whiE* (*whiEI*, *whiEVII*, and *whiEVIII*), *whiH*, *whiI*, *whiG*, *ku*, and *ligD*. The qRT-PCR results matched the microarray profiles, effectively validating our microarray data (Fig. S6a and 6b).

In the 16.5-day spaceflight samples, most of the differentially expressed involved in morphological differentiation and development were down-expressed, and a higher number of the genes were differentially regulated as compared to the 1-g control than to the S-1 g control. Genes for the BldK ABC transporter complex (*bldKB*, *bldKC*, and *bldKD*) were differentially expressed (upregulated) only in the case of 16.5-day  $\mu$ g versus 1 g. And, none of the *whi* genes were differentially expressed in the case of 16.5-day  $\mu$ g versus S-1 g, while *whiA* and *whiG* were upregulated and *whiEIII* and *sigF* were downregulated in the case of 16.5-day  $\mu$ g versus 1 g. These gene expression data were also confirmed by qRT-PCR results (Fig. S6c and 6d).

#### Effects of SMG and spaceflight on genes involved in antibiotic biosynthesis

Gene expression FCs from microarray results of the *act* gene cluster (SCO5071–5092) are shown in Table 2. According to



**Table 1** Gene expression FCs from microarray results of the 16 *whi* genes

Locus tag	Gene	Functional category	Fold change			
			4-d SMG vs 1 g	6-d SMG vs 1 g	16.5-d $\mu$ g vs S-1 g	16.5-d $\mu$ g vs 1 g
SCO1950	<i>whiA</i>	Aerial hyphae erection and sporulation	1.43	1.33	1.23	1.84
SCO3034	<i>whiB</i>	Aerial hyphae erection and sporulation	1.12	1.23	-1.24	-1.27
SCO5621	<i>whiG</i>	Aerial hyphae erection and sporulation	1.01	1.14	1.48	1.82
SCO5819	<i>whiH</i>	Aerial hyphae erection and sporulation	1.73	1.60	1.01	1.10
SCO6029	<i>whiI</i>	Aerial hyphae erection and sporulation	1.66	1.50	1.10	-1.19
SCO4543	<i>whiJ</i>	Aerial hyphae erection and sporulation	1.02	-1.39	-1.16	1.17
SCO4767	<i>whiD</i>	Sporulation	<b>3.90</b>	1.82	1.33	1.20
SCO5320	<i>whiEI</i>	Sporulation	<b>6.86</b>	<b>2.87</b>	-1.00	-1.19
SCO5319	<i>whiEII</i>	Sporulation	<b>2.59</b>	<b>2.80</b>	-1.28	-1.20
SCO5318	<i>whiEIII</i>	Sporulation	<b>2.21</b>	1.96	-1.10	<b>-2.04</b>
SCO5317	<i>whiEIV</i>	Sporulation	<b>4.02</b>	<b>2.10</b>	-1.04	-1.28
SCO5316	<i>whiEV</i>	Sporulation	<b>5.89</b>	<b>3.20</b>	1.07	1.13
SCO5315	<i>whiEVI</i>	Sporulation	<b>5.42</b>	<b>4.01</b>	1.17	1.12
SCO5314	<i>whiEVII</i>	Sporulation	<b>4.92</b>	<b>2.76</b>	1.04	-1.17
SCO5321	<i>whiEVIII</i>	Sporulation	1.19	1.31	-1.02	-1.31
SCO4035	<i>sigF</i>	Sporulation	<b>4.85</b>	1.80	-1.09	-1.99

FCs  $\geq 2.0$  are shown in bold

the microarray analysis in the case of SMG versus 1 g, transcription of the whole 22 genes of the ACT biosynthetic gene cluster exhibited a downregulation trend at day 4, with the genes *actVI-ORF3* (SCO5074), *actII-ORF2* (SCO5083), and *actII-ORF3* (SCO5084) downregulated over 2.0-fold, and exhibited obvious downregulation at day 6, with 20 genes downregulated over 2.0-fold and the other two genes (*actII-ORF4* and *actVB*) downregulated over 1.5-fold (Table 2). Interestingly, at day 6, most of the late genes of ACT biosynthesis (*actVA* and *actVI*, involving the biosynthesis of ACT end-product) were altered more substantially than the early-to-mid genes (*actI*, *actIII*, *actIV*, and *actVII*, involving the biosynthesis of ACT backbone). In the 16.5-day spaceflight samples, genes in the central regulatory region (*actII-ORF1* to *ORF4*) of the ACT cluster, except *actII-ORF4*, still showed a downregulation trend, and a couple of tailoring enzyme genes were upregulated as compared to the controls (Table 2).

In contrast, transcription of the whole 22 genes of the RED biosynthetic gene cluster (SCO5877-5898) exhibited no significant difference at days 4 and 6 in SMG, and only polyketide synthase gene *redX* (SCO5878) and thioesterase gene *redJ* (SCO5894) were differentially expressed over 2.0-fold in the 16.5-day spaceflight samples as compared to the controls (Table S3). The acyl carrier protein gene *redQ* was slightly downregulated ( $1.5 < FC < 2.0$ ) in the cases of 6-day SMG versus 1 g and 16.5-day  $\mu$ g versus 1 g. The transcription of

pathway-specific regulatory genes *redD* (SCO5877) and *redZ* (SCO5881) was not significantly altered in every case (Table S3).

The transcription profiles of representative genes *actII-ORF2*, *actII-ORF3*, *actII-ORF4*, *redD*, *redZ*, and *redQ* in each case were further examined by qRT-PCR, and the results matched our microarray profiles (Fig. S6).

Besides ACT and RED, expression of the gene clusters for another three antibiotics in *S. coelicolor* A3(2), CDA, MMY, and CPK, which have antimicrobial activity, were also analyzed, and the results are shown in Tables S4, S5, and S6, respectively. There were almost no significant differences at transcriptional level of the whole *cda* gene cluster (SCO3210-SCO3249) under either SMG or spaceflight microgravity, and ten out of the 40 genes were downregulated over 2.0-fold in the case of 16.5-day  $\mu$ g versus 1 g, including those involved in two-component system (TCS) (*absA1/absA2*) and ABC transporter (*SCE8.16c* and *SCE8.17c*) (Table S4). Transcription of the *mmv* gene cluster (SCO1.228c-SCP1.246) showed a decrease in the case of 6-day SMG versus 1 g, in that nine out of the 21 genes were downregulated over 2.0-fold (Table S5), which were mainly involved in biosynthetic process (*mmvE*, *mmvG*, *mmvO*, and *mmvT*) and regulation (*mmvB* and *mmvR*), but showed almost no significant differences in the other three cases. For CPK, seven out of the 20 genes in the *cpk* cluster (SCO6269-6288) were significantly upregulated at day 4 under SMG, including CPK biosynthetic enzyme genes (*cpkD*, *cpkE*, *cpkG*, *cpkI*, *cpkJ*, and *cpkK*) and CPK transporter gene

**Table 2** Gene expression FCs from microarray results of the *act* gene cluster (SCO5071-SCO5092)

Locus tag	Gene	Product	Fold change			
			4-d SMG vs 1 g	6-d SMG vs 1 g	16.5-d $\mu$ g vs S-1 g	16.5-d $\mu$ g vs 1 g
SCO5071	<i>actVI-ORFA</i>	Hydroxylacyl-CoA dehydrogenase	-1.62	<b>-3.73</b>	-1.06	1.62
SCO5072	<i>actVI-ORF1</i>	Hydroxylacyl-CoA dehydrogenase	-1.90	<b>-4.09</b>	-1.09	1.23
SCO5073	<i>actVI-ORF2</i>	Oxidoreductase	-1.80	<b>-4.62</b>	1.19	1.81
SCO5074	<i>actVI-ORF3</i>	Dehydratase	<b>-2.10</b>	<b>-4.40</b>	-1.12	1.60
SCO5075	<i>actVI-ORF4</i>	Oxidoreductase	-1.59	<b>-4.04</b>	1.55	<b>2.90</b>
SCO5076	<i>actVA-ORF1</i>	Integral membrane protein	-1.35	<b>-3.22</b>	1.21	1.49
SCO5077	<i>actVA-ORF2</i>	Hypothetical protein	-1.55	<b>-2.55</b>	-1.05	1.67
SCO5078	<i>actVA-ORF3</i>	Hypothetical protein	-1.53	<b>-4.63</b>	-1.06	1.20
SCO5079	<i>actVA-ORF4</i>	Hypothetical protein	-1.54	<b>-4.87</b>	-1.00	1.35
SCO5080	<i>actVA-ORF5</i>	Hydrolase	-1.55	<b>-5.39</b>	1.49	<b>2.05</b>
SCO5081	<i>actVA-ORF6</i>	Hypothetical protein	-1.37	<b>-4.41</b>	1.50	1.65
SCO5082	<i>actII-ORF1</i>	Transcriptional regulatory protein	-1.70	<b>-2.70</b>	-1.31	-1.04
SCO5083	<i>actII-ORF2</i>	Actinorhodin transporter	<b>-2.24</b>	<b>-3.55</b>	-1.75	-1.11
SCO5084	<i>actII-ORF3</i>	Hypothetical protein	<b>-2.23</b>	<b>-3.30</b>	<b>-2.05</b>	-1.49
SCO5085	<i>actII-ORF4</i>	Actinorhodin cluster activator protein	-1.37	-1.90	-1.03	1.66
SCO5086	<i>actIII</i>	Ketoacylreductase	-1.25	<b>-2.46</b>	-1.00	1.33
SCO5087	<i>actI-ORF1</i>	Actinorhodin polyketide beta-ketoacyl synthase subunit alpha	-1.71	<b>-2.80</b>	1.18	1.50
SCO5088	<i>actI-ORF2</i>	Actinorhodin polyketide beta-ketoacyl synthase subunit beta	-1.03	<b>-2.74</b>	-1.01	-1.14
SCO5089	<i>actI-ORF3</i>	Actinorhodin polyketide synthase acyl carrier protein	-1.28	<b>-3.12</b>	1.30	1.63
SCO5090	<i>actVII</i>	Actinorhodin polyketide synthase bifunctional cyclase/dehydratase	-1.95	<b>-3.69</b>	1.54	<b>2.05</b>
SCO5091	<i>actIV</i>	Cyclase	-1.14	<b>-2.68</b>	1.47	1.59
SCO5092	<i>actVB</i>	Actinorhodin polyketide dimerase	-1.23	-1.66	1.36	1.21

FCs  $\geq 2.0$  are shown in bold

(*cpkF*); three genes (*cpkJ*, *cpkK*, and *cpkL*) were upregulated in the case of 16.5-day  $\mu$ g versus 1 g; and no significant differences were found in the cases of 6-day SMG versus 1 g or 16.5-day  $\mu$ g versus S-1 g (Table S6).

## Discussion

With the human exploration of space accelerating, the growth, development, and metabolic responses of microorganisms to the extreme environment of space are receiving increasing concerns. As streptomycetes have a complex life cycle and are the richest source of medically useful natural products, notably antibiotics, exploring the response of streptomycetes to microgravity environment not only allows for understanding the growth and development of filamentous microorganisms in the normal Earth gravity, but is also important for utilizing space resources (microgravity and radiation) to produce natural drugs. In this study, we investigated the effects of long-term (16.5 days) spaceflight on *S. coelicolor* subjected to the Shenzhou-8 space mission. It cannot be ignored that,

during spaceflight, some factors other than microgravity could also have effects on microorganisms, such as radiation, vibration generated by rocket, and acceleration during launch and landing of the spacecraft; however, our experiment successfully eliminated the interference factors by using the simulated 1-g control in space (S-1 g). Due to technical limitations, on-orbit fixation was not carried out on microorganisms during the mission; so, we were not able to obtain direct evidence for the short-term effects of spaceflight on *S. coelicolor*. Nevertheless, before and after the mission, we analyzed the short-term (<8 days) and long-term (16.5 days) alterations in *S. coelicolor* A3(2) exposed to clinostat-SMG on ground, in consideration of the life cycle period of streptomycetes and in coordination with the spaceflight duration of Shenzhou-8 mission. Our results showed that the effects of spaceflight on *S. coelicolor* were largely in accordance with the SMG effects and revealed for the first time that microgravity accelerates morphological differentiation process and alters secondary metabolism of streptomycetes.

The cultural characteristics and microscopic morphological observations suggested that the process from aerial hyphae to mature spores (sporulation) of *S. coelicolor* on agar was

accelerated under microgravity conditions. Moreover, the much more fragmented, thicker, and well-dispersed mycelium in liquid suspension cultures at  $\mu\text{g}$  position on-orbit was unique among all samples (Fig. 4), indicating that the size and shape of *S. coelicolor* mycelium in liquid medium were also affected by space microgravity.

The microarray data confirmed that morphological differentiation and development of *S. coelicolor* were susceptible to microgravity conditions. Spore pigmentation in *S. coelicolor* A3(2) depends on the production of a polycyclic aromatic polyketide (TW95a) during the maturation of the spores, which is specified by a complex locus *whiE*, and the *whiE* transcription is regulated positively by *whiD*, *sigF*, *whiH*, and *whiI* (Davis and Chater 1990; Kelemen et al. 1998). The *whiH* and *whiI* are “early-to-mid” *whi* genes required for aerial hyphae septum formation at the early stage of sporulation (Aínsa et al. 1999; Ryding et al. 1998), and the *whiD*, *sigF*, and *whiE* are “late” *whi* genes required for spore maturation and pigmentation at the late stage of sporulation (Kelemen et al. 1998; Molle et al. 2000). In our study, the upregulation of all these five gene loci during days 4–6 of solid cultivation under SMG conditions is consistent with the phenotypes of accelerated morphological differentiation and increased spore pigment accumulation. Moreover, the significant upregulation of *whiD*, *sigF*, and *whiE* on the 4th day in SMG clearly indicates that the development of *S. coelicolor* A3(2) has been switched into the late stage of sporulation ahead of normal schedule. It is reasonable that no differences were detected in the expression of the other four “early-to-mid” *whi* genes, *whiG*, *whiJ*, *whiA*, and *whiB*, which are even earlier than the *whiH* and *whiI* (Chater 1998), because *S. coelicolor* A3(2) had entered into late life cycle stages after 4 days (Fig. 1). A previous study also showed that *whiG* might be regulated posttranscriptionally and its transcription was constant in the whole development process (Kelemen et al. 1996). The upregulation of most of the other differentially expressed genes involved in the development of *S. coelicolor* in SMG conditions further supports at the gene transcriptional level that morphological differentiation of *S. coelicolor* A3(2) was accelerated under SMG. In the 16.5-day samples, the sporulation of *S. coelicolor* A3(2) had completed; thus, the *whi* genes showed no difference in expression (16.5-day  $\mu\text{g}$  vs S-1 g). The upregulation (*whiA* and *whiG*) and downregulation (*sigF* and *whiEIII*) of some *whi* genes in the case of 16.5-day  $\mu\text{g}$  versus 1 g, plus the upregulation of “early” genes of *bldK*, might indicate the initiation of another round of aerial hyphal growth on the old *S. coelicolor* colonies in space, possibly due to some other space environmental factors, for example, cosmic radiation. By integration of the morphological, chemical, microarray, and qRT-PCR data, we conclude that the complex life cycle of *S. coelicolor* A3(2) is shorten under both SMG and spaceflight conditions.

Our study indicated that the growth rate of *S. coelicolor* A3(2) cultured on yeast-starch agar was not influenced by

either SMG or spaceflight, but the cell biomass of liquid cultures was increased under both conditions. These results are similar to the previous studies on *E. coli* and *B. subtilis*, which also showed that the latter two microorganisms grew faster and yielded more biomass in liquid suspension cultures during spaceflight (Kacena et al. 1999; Klaus et al. 1997; Mennigmann and Lange 1986), but exhibited no visible difference in growth rate in agar or semi-solid cultures (Kacena and Todd 1997; Kacena et al. 1997). It was speculated that the growth rate of strains was related to fluid mechanics and the distribution of liquid medium, rather than to cellular effects induced by microgravity environment (Kacena et al. 1997), so a potential explanation of our observation that *S. coelicolor* A3(2) liquid cultures grew faster in SMG and spaceflight is a reduced requirement for culture mixing to equalize nutrient levels throughout the culture volume.

The observation of stronger bacteriostatic activity against *B. subtilis* possibly indicates that *S. coelicolor* A3(2) could produce more bioactive substances under microgravity conditions. It has been reported that *S. coelicolor* A3(2) produces four anti-*B. subtilis* products, ACT (with weak activity) (Wright and Hopwood 1976), CDA (Lakey et al. 1983), MMY (Haneishi et al. 1974), and a yellow-pigmented antibiotic yCPK or its colorless precursor abCPK (Gottelt et al. 2010). Because both chemical and microarray analyses revealed that the biosynthesis of ACT was declined under SMG and spaceflight, and microarray analysis showed either no significant induction or downregulation of the *cda* and *mmv* gene clusters under the conditions, it is unlikely that the enhanced bioactivity was derived from ACT, CDA, or MMY. On the other hand, the significant upregulation of some CPK biosynthetic enzyme genes and a transporter gene (*cpkF*) at day 4 under SMG suggested that the enhanced bioactivity might be contributed by-products of the *cpk* gene cluster, yCPK, and/or abCPK, which are cryptic type I polyketides that emerge during mid-transition phase of growth and are difficult to detect (Gottelt et al. 2010; Pawlik et al. 2007). Furthermore, as we did not observe any yellow pigment during our experiments, we speculate that the enhanced activity of *S. coelicolor* A3(2) against *B. subtilis* under microgravity may be ascribed to the colorless abCPK, while other unknown bioactive compounds produced by *S. coelicolor* A3(2) cannot be excluded.

Our phenotypic and transcriptomic data indicate that microgravity conditions also alter secondary metabolism of *S. coelicolor* A3(2); however, the effects vary among different pathways. For example, the production of the only two type II polyketides, spore pigment and ACT, as well as the transcription of their gene clusters responded contrary to both spaceflight and SMG. It has been demonstrated that biochemical “cross-talk” between these two PKS (i.e., *whiE*-PKS and *act*-PKS) is normally prevented due to a differential spatial localization of expression of their gene sets (Yu and Hopwood 1995); so, the increase of *whiE*-PKS could not complement



the decrease of its *act*-PKS homolog but consumed more substrates shared with the latter. In addition, as antibiotic biosynthesis in streptomycetes is also controlled by global regulators, the upregulation of the global regulatory gene *sigQ*, which works together with the TCS *afsQ1/afsQ2*, could inhibit the biosynthesis of ACT, RED, and CDA (Shu et al. 2009), while the downregulation of some other negative regulators, such as the TCS response regulator SCO0204 (Wang et al. 2009) under SMG (4-d, FC=-7.46; 6-d, FC=-1.53) and spaceflight microgravity (16.5-d  $\mu$ g vs S-1 g, FC=-1.64), might stimulate the antibiotic biosynthesis. Previous studies of streptomycete secondary metabolism under microgravity also showed that productions of some antibiotics decreased, such as cephalosporin C by *S. clavuligerus* and rapamycin by *S. hygroscopicus* in RWBs under SMG (Fang et al. 2000; Fang et al. 1997), while some antibiotics increased, such as actinomycin D by *S. plicatus* during spaceflight (Benoit et al. 2006; Lam et al. 2002). From this and previous studies, and considering the complex regulatory network of antibiotic production, it is likely that the alteration of microgravity to secondary metabolism of streptomycetes is pathway- and/or case-specific, lacking directed and consistent behavior.

As the clinostat-SMG on ground is not equal to the real microgravity in space and is based on the principle of the minimum response time (MRT) of organisms to the continuous changing direction of the gravity vector, the short-term effects of SMG in our results might not all be the same as the short-term effects of microgravity by spaceflight. Nevertheless, to our knowledge, this is the first report on the responses of morphological differentiation and secondary metabolism of filamentous bacteria, streptomycetes, to microgravity at both the phenotypic and whole transcriptome levels, which could pave the way for future microgravity studies on this kind of promising microorganisms, in well-equipped spacecrafts or the Space Station.

**Acknowledgments** This work was supported by the China Manned Space Engineering Program (CMSE, 921-2). We thank Prof. Hua-Rong Tan (Institute of Microbiology, CAS) for kindly providing *S. coelicolor* A3(2) and M145, and Chunli Li and Jingnan Liang (Institute of Microbiology, CAS) for their help in SEM analyses. We are also grateful to German Aerospace Center's (DLR) Space Administration, EADS (Astrium), the China Manned Space Engineering Office (CMSEO; now CMSA, China Manned Space Agency) and the General Establishment of Space Science and Application, Chinese Academy of Sciences (GESSA, CAS) for technical and logistical support to the SIMBOX-Shenzhou-8 space mission.

## References

- Ainsa JA, Parry HD, Chater KF (1999) A response regulator-like protein that functions at an intermediate stage of sporulation in *Streptomyces coelicolor* A3(2). *Mol Microbiol* 34:607–619. doi:10.1046/j.1365-2958.1999.01630.x
- Benoit MR, Klaus DM (2007) Microgravity, bacteria, and the influence of motility. *Adv Space Res* 39:1225–1232. doi:10.1089/ast.2010.0536
- Benoit MR, Li W, Stodieck LS, Lam KS, Winther CL, Roane TM, Klaus DM (2006) Microbial antibiotic production aboard the International Space Station. *Appl Microbiol Biotechnol* 70:403–411. doi:10.1007/s00253-005-0098-3
- Bentley SD, Chater KF, Cerdeno-Tarraga AM, Challis GL, Thomson NR, James KD, Harris DE, Quail MA, Kieser H, Harper D, Bateman A, Brown S, Chandra G, Chen CW, Collins M, Cronin A, Fraser A, Goble A, Hidalgo J, Hornsby T, Howarth S, Huang CH, Kieser T, Larke L, Murphy L, Oliver K, O'Neil S, Rabinowitsch E, Rajandream MA, Rutherford K, Rutter S, Seeger K, Saunders D, Sharp S, Squares R, Squares S, Taylor K, Warren T, Wietzorrek A, Woodward J, Barrell BG, Parkhill J, Hopwood DA (2002) Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). *Nature* 417:141–147. doi:10.1038/417141a
- Bibb MJ (2005) Regulation of secondary metabolism in streptomycetes. *Curr Opin Microbiol* 8:208–215. doi:10.1016/j.mib.2005.02.016
- Brungs S, Hauslage J, Hilbig R, Hemmersbach R, Anken R (2011) Effects of simulated weightlessness on fish otolith growth: clinostat versus rotating-wall vessel. *Adv Space Res* 48:792–798. doi:10.1016/j.asr.2011.04.014
- Chater KF (1998) Taking a genetic scalpel to the *Streptomyces* colony. *Microbiol-SGM* 144:1465–1478. doi:10.1099/00221287-144-6-1465
- Crabbé A, De Boever P, Van Houdt R, Moors H, Mergeay M, Cornelis P (2008) Use of the rotating wall vessel technology to study the effect of shear stress on growth behaviour of *Pseudomonas aeruginosa* PAO1. *Environ Microbiol* 10:2098–2110. doi:10.1111/j.1462-2920.2008.01631.x
- Crabbé A, Pycke B, Van Houdt R, Monsieurs P, Nickerson C, Leys N, Cornelis P (2010) Response of *Pseudomonas aeruginosa* PAO1 to low shear modelled microgravity involves AlgU regulation. *Environ Microbiol* 12:1545–1564. doi:10.1111/j.1462-2920.2010.02184.x
- Crabbé A, Schurr MJ, Monsieurs P, Morici L, Schurr J, Wilson JW, Ott CM, Tsapralis G, Pierson DL, Stefanyshyn-Piper H, Nickerson CA (2011) Transcriptional and proteomic responses of *Pseudomonas aeruginosa* PAO1 to spaceflight conditions involve Hfq regulation and reveal a role for oxygen. *Appl Environ Microbiol* 77:1221–1230. doi:10.1128/Aem.01582-10
- Davis NK, Chater KF (1990) Spore color in *Streptomyces coelicolor* A3(2) involves the developmentally regulated synthesis of a compound biosynthetically related to polyketide antibiotics. *Mol Microbiol* 4:1679–1691. doi:10.1111/j.1365-2958.1990.tb00545.x
- Demain AL, Fang A (2001) Secondary metabolism in simulated microgravity. *Chem Rec* 1:333–346. doi:10.1002/Tcr.1018
- Eiermann P, Kopp S, Hauslage J, Hemmersbach R, Gerzer R, Ivanova K (2013) Adaptation of a 2-D clinostat for simulated microgravity experiments with adherent cells. *Microgravity Sci Technol* 25: 153–159. doi:10.1007/s12217-013-9341-1
- Fang A, Pierson DL, Mishra SK, Koenig DW, Demain AL (1997) Secondary metabolism in simulated microgravity:  $\beta$ -lactam production by *Streptomyces clavuligerus*. *J Ind Microbiol Biotechnol* 18: 22–25. doi:10.1038/sj.jim.2900345
- Fang A, Pierson DL, Mishra SK, Demain AL (2000) Growth of *Streptomyces hygroscopicus* in rotating-wall bioreactor under simulated microgravity inhibits rapamycin production. *Appl Microbiol Biotechnol* 54:33–36. doi:10.1007/s002539900303
- Flardh K, Buttner MJ (2009) *Streptomyces* morphogenetics: dissecting differentiation in a filamentous bacterium. *Nat Rev Microbiol* 7:36–49. doi:10.1038/Nrmicro1968
- Gao C, Hindra MD, Yin C, Elliot MA (2012) Crp is a global regulator of antibiotic production in *Streptomyces*. *MBio* 3:e00407–e00412. doi:10.1128/mBio.00407-12

- Gottelt M, Kol S, Gomez-Escribano JP, Bibb M, Takano E (2010) Deletion of a regulatory gene within the *cpk* gene cluster reveals novel antibacterial activity in *Streptomyces coelicolor* A3(2). *Microbiol-SGM* 156:2343–2353. doi:10.1099/Mic.0.038281-0
- Haneishi T, Kitahara N, Takiguchi Y, Arai M, Sugawara S (1974) New antibiotics, methylenomycins A and B. I. Producing organism, fermentation and isolation, biological-activities and physical and chemical properties. *J Antibiot* 27:386–392. doi:10.7164/antibiotics.27.386
- Hemmersbach R, Strauch SM, Seibt D, Schuber M (2006) Comparative studies on gravisensitive protists on ground (2D and 3D clinostats) and in microgravity. *Microgravity Sci Technol* 18:257–259. doi:10.1007/Bf02870423
- Herranz R, Anken R, Boonstra J, Braun M, Christianen PC, de Geest M, Hauslage J, Hilbig R, Hill RJ, Lebert M, Medina FJ, Vagt N, Ullrich O, van Loon JJ, Hemmersbach R (2013) Ground-based facilities for simulation of microgravity: organism-specific recommendations for their use, and recommended terminology. *Astrobiology* 13:1–17. doi:10.1089/ast.2012.0876
- Kacena MA, Todd P (1997) Growth characteristics of *Escherichia coli* and *Bacillus subtilis* cultured on an agar substrate in microgravity. *Microgravity Sci Technol* 10:58–62
- Kacena MA, Leonard PE, Todd P, Lutges MW (1997) Low gravity and inertial effects on the growth of *Escherichia coli* and *Bacillus subtilis* in semi-solid media. *Aviat Space Environ Med* 68:1104–1108
- Kacena MA, Merrell GA, Manfredi B, Smith EE, Klaus DM, Todd P (1999) Bacterial growth in space flight: logistic growth curve parameters for *Escherichia coli* and *Bacillus subtilis*. *Appl Microbiol Biotechnol* 51:229–234. doi:10.1007/s002530051386
- Kang SG, Jin W, Bibb M, Lee KJ (1998) Actinorhodin and undecylprodigiosin production in wild-type and *relA* mutant strains of *Streptomyces coelicolor* A3(2) grown in continuous culture. *FEMS Microbiol Lett* 168:221–226. doi:10.1016/S0378-1097(98)00446-7
- Kelemen GH, Brown GL, Kormanec J, Potuckova L, Chater KF, Buttner MJ (1996) The positions of the sigma-factor genes, *whiG* and *sigF*, in the hierarchy controlling the development of spore chains in the aerial hyphae of *Streptomyces coelicolor* A3(2). *Mol Microbiol* 21:593–603. doi:10.1111/j.1365-2958.1996.tb02567.x
- Kelemen GH, Brian P, Flårdh K, Chamberlin L, Chater KF, Buttner MJ (1998) Developmental regulation of transcription of *whiE*, a locus specifying the polyketide spore pigment in *Streptomyces coelicolor* A3(2). *J Bacteriol* 180:2515–2521
- Kirby R, Herron P, Hoskisson P (2011) Analysis of developmental gene conservation in the Actinomycetales using DNA/DNA microarray comparisons. *Anton Leeuw Int J G* 99:159–177. doi:10.1007/s10482-010-9473-x
- Klaus DM (2001) Clinostats and Bioreactors. *Gravit Space Biol Bull* 14(2):55–64
- Klaus D, Simske S, Todd P, Stodieck L (1997) Investigation of space flight effects on *Escherichia coli* and a proposed model of underlying physical mechanisms. *Microbiol-SGM* 143:449–455. doi:10.1099/00221287-143-2-449
- Lakey JH, Lea EJA, Rudd BAM, Wright HM, Hopwood DA (1983) A new channel-forming antibiotic from *Streptomyces coelicolor* A3(2) which requires calcium for its activity. *J Gen Microbiol* 129:3565–3573. doi:10.1099/00221287-129-12-3565
- Lam KS, Mamber SW, Pack EJ, Forenza S, Fernandes PB, Klaus DM (1998) The effects of space flight on the production of monorden by *Humicola fuscoatra* WC5157 in solid-state fermentation. *Appl Microbiol Biotechnol* 49:579–583. doi:10.1007/s002530051216
- Lam KS, Gustavson DR, Pimik DL, Pack E, Bulanahagui C, Mamber SW, Forenza S, Stodieck LS, Klaus DM (2002) The effect of space flight on the production of actinomycin D by *Streptomyces plicatus*. *J Ind Microbiol Biotechnol* 29:299–302. doi:10.1038/sj.jim.7000312
- Lawal A, Jejelowo OA, Rosenzweig JA (2010) The effects of low-shear mechanical stress on *Yersinia pestis* virulence. *Astrobiology* 10:881–888. doi:10.1089/ast.2010.0493
- Lawal A, Kirtley ML, van Lier CJ, Erova TE, Kozlova EV, Sha J, Chopra AK, Rosenzweig JA (2013) The effects of modeled microgravity on growth kinetics, antibiotic susceptibility, cold growth, and the virulence potential of a *Yersinia pestis ymoA*-deficient mutant and its isogenic parental strain. *Astrobiology* 13:821–832. doi:10.1089/ast.2013.0968
- Liu G, Chater KF, Chandra G, Niu GQ, Tan HR (2013) Molecular regulation of antibiotic biosynthesis in *Streptomyces*. *Microbiol Mol Biol Rev* 77:112–143. doi:10.1128/Mmbr.00054-12
- Lynch SV, Brodie EL, 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 SV, Mukundakrishnan K, Benoit MR, Ayyaswamy PS, Matin A (2006) *Escherichia coli* biofilms formed under low-shear modeled microgravity in a ground-based system. *Appl Environ Microbiol* 72:7701–7710. doi:10.1128/Aem.01294-06
- Mennigmann HD, Lange M (1986) Growth and differentiation of *Bacillus subtilis* under microgravity. *Naturwissenschaften* 73:415–417. doi:10.1007/Bf00367283
- Molle V, Palfaman WJ, Findlay KC, Buttner MJ (2000) WhiD and WhiB, homologous proteins required for different stages of sporulation in *Streptomyces coelicolor* A3(2). *J Bacteriol* 182:1286–1295. doi:10.1128/Jb.182.5.1286-1295.2000
- Nasir A, Strauch SM, Becker I, Sperling A, Schuster M, Richter PR, Weisskopf M, Ntefidou M, Daiker V, An YA, Li XY, Liu YD, Lebert M (2014) The influence of microgravity on *Euglena gracilis* as studied on Shenzhou 8. *Plant Biol* 16:113–119. doi:10.1111/plb.12067
- Paulsen K, Tauber S, Goelz N, Simmet DM, Engeli S, Birlem M, Dumrese C, Karer A, Hunziker S, Biskup J, Konopasek S, Suh D, Hurlimann E, Signer C, Wang A, Sang C, Grote KH, Zhuang FY, Ullrich O (2014) Severe disruption of the cytoskeleton and immunologically relevant surface molecules in a human macrophageal cell line in microgravity—Results of an in vitro experiment on board of the Shenzhou-8 space mission. *Acta Astronaut* 94:277–292. doi:10.1016/j.actaastro.2013.06.007
- Pawlik K, Kotowska M, Chater KF, Kuczek K, Takano E (2007) A cryptic type I polyketide synthase (*cpk*) gene cluster in *Streptomyces coelicolor* A3(2). *Arch Microbiol* 187:87–99. doi:10.1007/s00203-006-0176-7
- Pietsch J, Ma X, Wehland M, Aleshcheva G, Schwarzwald A, Segerer J, Birlem M, Horn A, Bauer J, Infanger M, Grimm D (2013) Spheroid formation of human thyroid cancer cells in an automated culturing system during the Shenzhou-8 Space mission. *Biomaterials* 34:7694–7705. doi:10.1016/j.biomaterials.2013.06.054
- Prasad G, Jayaram S, Ward J, Gupta P (2004) SimBOX: a scalable architecture for aggregate distributed command and control of spaceport and service constellation. *Proc SPIE Enabling Technol Simul Sci* 5423(VIII):437–446. doi:10.1117/12.543058
- Preu P, Braun M (2014) German SIMBOX on Chinese mission Shenzhou-8: Europe's first bilateral cooperation utilizing China's Shenzhou programme. *Acta Astronaut* 94:584–591. doi:10.1016/j.actaastro.2013.08.022
- Ryding NJ, Kelemen GH, Whatling CA, Flårdh K, Buttner MJ, Chater KF (1998) A developmentally regulated gene encoding a repressor-like protein is essential for sporulation in *Streptomyces coelicolor* A3(2). *Mol Microbiol* 29:343–357. doi:10.1046/j.1365-2958.1998.00939.x
- Shu D, Chen L, Wang WH, Yu ZY, Ren C, Zhang WW, Yang S, Lu YH, Jiang WH (2009) *afsQ1-Q2-sigQ* is a pleiotropic but conditionally required signal transduction system for both secondary metabolism and morphological development in *Streptomyces coelicolor*. *Appl*

- Microbiol Biotechnol 81:1149–1160. doi:10.1007/s00253-008-1738-1
- Thiel CS, Paulsen K, Bradacs G, Lust K, Tauber S, Dumrese C, Hilliger A, Schoppmann K, Biskup J, Golz N, Sang C, Ziegler U, Grote KH, Zipp F, Zhuang FY, Engelmann F, Hemmersbach R, Cogoli A, Ullrich O (2012) Rapid alterations of cell cycle control proteins in human T lymphocytes in microgravity. *Cell Commun Signal* 10:1–16. doi:10.1186/1478-811x-10-1
- van Loon JJWA (2007) Some history and use of the random positioning machine, RPM, in gravity related research. *Adv Space Res* 39:1161–1165. doi:10.1016/j.asr.2007.02.016
- Van Mulders SE, Stassen C, Daenen L, Devreese B, Siewers V, van Eijsden RG, Nielsen J, Delvaux FR, Willaert R (2011) The influence of microgravity on invasive growth in *Saccharomyces cerevisiae*. *Astrobiology* 11:45–55. doi:10.1089/ast.2010.0518
- Viollier PH, Kelemen GH, Dale GE, Nguyen KT, Buttner MJ, Thompson CJ (2003) Specialized osmotic stress response systems involve multiple SigB-like sigma factors in *Streptomyces coelicolor*. *Mol Microbiol* 47:699–714. doi:10.1046/j.1365-2958.2003.03302.x
- Wang WH, Shu D, Chen L, Jiang WH, Lu YH (2009) Cross-talk between an orphan response regulator and a noncognate histidine kinase in *Streptomyces coelicolor*. *FEMS Microbiol Lett* 294:150–156. doi:10.1111/j.1574-6968.2009.01563.x
- Wilson JW, Ott CM, Ramamurthy R, Porwollik S, McClelland M, Pierson DL, Nickerson CA (2002a) Low-shear modeled microgravity alters the *Salmonella enterica* serovar typhimurium stress response in an RpoS-independent manner. *Appl Environ Microbiol* 68:5408–5416. doi:10.1128/Aem.68.11.5408-5416.2002
- Wilson JW, Ramamurthy R, Porwollik S, McClelland M, Hammond T, Allen P, Ott CM, Pierson DL, Nickerson CA (2002b) Microarray analysis identifies *Salmonella* genes belonging to the low-shear modeled microgravity regulon. *Proc Natl Acad Sci U S A* 99:13807–13812. doi:10.1073/pnas.212387899
- Wilson JW, Ott CM, Bentrup KHZ, Ramamurthy R, Quick L, Porwollik S, Cheng P, McClelland M, Tsapraillis G, Radabaugh T, Hunt A, Fernandez D, Richter E, Shah M, Kilcoyne M, Joshi L, Neiman-Gonzalez M, Hing S, Parra M, Dumars P, Norwood K, Bober R, Devich J, Ruggles A, Goulart C, Rupert M, Stodieck L, Stafford P, Catella L, Schurr MJ, Buchanan K, Morici L, McCracken J, Allen P, Baker-Coleman C, Hammond T, Vogel J, Nelson R, Pierson DL, Stefanyshyn-Piper HM, Nickerson CA (2007) Space flight alters bacterial gene expression and virulence and reveals a role for global regulator Hfq. *Proc Natl Acad Sci U S A* 104:16299–16304. doi:10.1073/pnas.0707155104
- Wright LF, Hopwood DA (1976) Actinorhodin is a chromosomally-determined antibiotic in *Streptomyces coelicolor* A3(2). *J Gen Microbiol* 96:289–297. doi:10.1099/00221287-96-2-289
- Yu TW, Hopwood DA (1995) Ectopic expression of the *Streptomyces coelicolor* *whiE* genes for polyketide spore pigment synthesis and their interaction with the *act* genes for actinorhodin biosynthesis. *Microbiol-SGM* 141:2779–2791. doi:10.1099/13500872-141-11-2779
- Yu TW, Shen YM, McDaniel R, Floss HG, Khosla C, Hopwood DA, Moore BS (1998) Engineered biosynthesis of novel polyketides from *Streptomyces* spore pigment polyketide synthases. *J Am Chem Soc* 120:7749–7759. doi:10.1021/Ja9803658