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ORIGINAL ARTICLE

Effects of Space Environment on Genome, Transcriptome, and Proteome of *Klebsiella pneumoniae*

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Background and Aims. The aim of this study was to explore the effects of space flight on *Klebsiella pneumoniae*.

Methods. A strain of *K. pneumoniae* was sent to space for 398 h aboard the ShenZhou VIII spacecraft during November 1, 2011—November 17, 2011. At the same time, a ground simulation with similar temperature conditions during the space flight was performed as a control. After the space mission, the flight and control strains were analyzed using phenotypic, genomic, transcriptomic and proteomic techniques.

Results. The flight strains LCT-KP289 exhibited a higher cotrimoxazole resistance level and changes in metabolism relative to the ground control strain LCT-KP214. After the space flight, 73 SNPs and a plasmid copy number variation were identified in the flight strain. Based on the transcriptomic analysis, there are 232 upregulated and 1879 downregulated genes, of which almost all were for metabolism. Proteomic analysis revealed that there were 57 upregulated and 125 downregulated proteins. These differentially expressed proteins had several functions that included energy production and conversion, carbohydrate transport and metabolism, translation, ribosomal structure and biogenesis, posttranslational modification, protein turnover, and chaperone functions. At a systems biology level, the *ytfG* gene had a synonymous mutation that resulted in significantly downregulated expression at both transcriptomic and proteomic levels.

Conclusions. The mutation of the *ytfG* gene may influence fructose and mannose metabolic processes of *K. pneumoniae* during space flight, which may be beneficial to the field of space microbiology, providing potential therapeutic strategies to combat or prevent infection in astronauts.

Key Words: Klebsiella pneumoniae, Space flight, Genome, Transcriptome, Proteome, *ytfG*.

Introduction

The spacecraft environment in space consists of microgravity, radiation and magnetic fields, etc. Astronauts living in this environment cannot avoid the presence of bacteria including bacteria carried by astronauts and/or the flight body compartment itself (1). More than 250 species of bacteria have been cultured from the Mir space station since it launched 15 years ago. Recent studies have shown that some bacteria in the space environment may be changed physiologically, biochemically, and genetically, which could increase the virulence and resistance of bacteria that were originally harmless to humans (2). Space research projects on *Streptococcus pneumoniae* were carried out in 2008 by the Unites States NASA Program (SPEGIS), which found that the state of low-shear modeled microgravity

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(LSMMG) increased *S. pneumoniae* adhesion and infection of respiratory epithelial cells. In a mouse-infection model, the median lethal dose (LD_{50}) of a microgravity-exposed strain was significantly decreased compared to the wild-type strain. Moreover, genetic analysis showed that LSMMG can induce some changes in gene expression in *S. pneumoniae* but does not influence the genes that encode the main virulence factor (3). In addition, simulated weightlessness altered the virulence and gene transcription of *Pseudomonas aeruginosa*, *Salmonella* and *E. coli*. However, the results of different studies are not consistent and a specific molecular mechanism has not been implicated (4−6).

K. pneumoniae is an opportunistic pathogen. It can migrate to several parts of the human including the lungs, urinary tract or blood, causing infection when immunity declines. Even if antibiotic therapy is given, *K. pneumonia*-infected patients still have a poor prognosis and the mortality rate remains as high as 20−54% (7). In addition, *K. pneumoniae* has been detected in astronauts who resided on the space station (8). Research into the extreme diversity of microbial organisms in space will lead to the identification of novel biological pathways and gene products and, thus, novel potential therapeutic strategies to combat or prevent infection in astronauts. However, there are limited opportunities for space flight experiments. No such study has investigated how *K. pneumoniae* adapts to microgravity and radiation in the space environment or how space exposure changes growth, pathogenicity, immunogenicity, and other relevant mechanisms. In this study we explore the changes of *K. pneumoniae* that occurred during approximately 398 h of space travel aboard the Shenzhou VIII spacecraft.

Materials and Methods

Bacterial Strains and Media

K. pneumoniae strains used in this study were *K. pneumoniae* CGMCC 1.1736, which were obtained from the China General Microbiological Culture Collection Center (CGMCC). Prior to the departure of the Shenzhou VIII spacecraft, one single colony of *K. pneumonia* strain 1.230 was inoculated in semi-solid Luria-Bertani (LB) medium in a plastic container designed for this mission. The LB medium per liter contained tryptone (10 g/L), yeast extract (5 g/L), NaCl (10 g/L) and agar (5 g/L). The pH of the medium was adjusted to 7.0−7.2. The plastic container was launched into space aboard the Shenzhou VIII spacecraft on November 1, 2011 and retrieved on November 16, 2011. In parallel, a ground control strain was also cultured as the space flight strain according to the variation of spaceship temperature. After the spaceflight returned to Earth, a mutant flight strain LCT-KP289 and a control strain LCT-KP214 were selected on the basis of phenotypic characteristics.

Phenotypic Analysis

To investigate the differences between the space-induced mutant strain (LCT-KP289) and the original strain (LCT-KP214), phenotype experiments were performed. Biochemical features were determined using the Biolog bacterial identification system (Biolog, Hayward, CA) according to the manufacturer's instructions. These are 96-well microtiter plates with a different cell culture medium dried to the bottom of each well. Media are designed to test unique cellular phenotypes. Electrons produced during respiration are transferred to an indicator dye, resulting in a dark purple color (9). Bacteria were grown overnight on LB agar. Standardized cell suspensions were produced using the 85% turbidity (T) standard, and 100 µl was added to each well. Growth was analyzed by observing the change of the microplate color at 37°C after 24 h. In addition, the first plate served as a blank test.

Antibiotic susceptibility tests performed to identify any organism that contributes to an infectious process warranting antimicrobial chemotherapy was carried out using the disk diffusion method (10). Mueller-Hinton agar plates were coated with the 100-µl suspension at a density of 10^7-10^8 CFU/ml. Antibiotic-impregnated disks were placed on the surface of the plate, and the diameter of the zone of inhibition around each disk was measured in a standard manner after incubation for 18−24 h at 37°C. Fourteen antibiotics including ampicillin, cefazolin, ceftazidime, ceftriaxone sodium, azithromycin, ciprofloxacin, the pediatric compound sulfamethoxazole, chloramphenicol, cefoperazone sodium, amikacin, streptomycin, minocycline, meropenem, and piperacillin/tazobactam were selected to test the resistance of the two strains. Each plate contained three types of antibiotics, and two tablets were used for each antibiotic.

Two strains were grown on LB liquid medium for 18 h at 37°C, ~20 µl suspensions were inoculated onto microtiter plates (honeycomb plates) containing 350 µl LB broth. The microtiter plate was detected using Bioscreen C (Lab Systems, Helsinki, Finland) at 37°C with continuous shaking. Each sample was measured for growth at an optical density at 600 nm (OD 600) in duplicate three times. A well with only 370 µl of LB was also included as negative control.

Genome Sequencing and Relevant Analysis

To obtain the whole genome information of these two strains of *K. pneumoniae*, two paired-end libraries (500 bp and 6 kb) were established. The whole genome shotgun sequencing was run on the Illumina HiSeq 2000 sequencer (Illumina, Inc., San Diego, CA) according to standard protocols. All read lengths were set to 90 base pairs (bp), and reads with