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ORIGINAL ARTICLE

Genomic and Proteomic Analysis of *Escherichia coli* After Spaceflight Reveals Changes Involving Metabolic Pathways

Xiaojun Zhang, Xiangqun Fang, and Changting Liu

Nanlou Respiratory Diseases Department, Chinese PLA General Hospital, Beijing, China Received for publication January 7, 2015; accepted March 25, 2015 (ARCMED-D-15-00014).

Background and Aims. The space environment could have impacts on a variety of characteristics of microorganism such as cell metabolism, drug resistance, and virulence. However, relevant mechanisms need to be clarified. In the present study, the effect of a space environment on Escherichia coli was investigated.

Methods. E. coli strains were sent to space for 398 h on the Shenzhou VIII and ground simulation was conducted as control. After the flight, a mutant strain LCT-EC67 was selected for further analysis.

Results. Although no changes in hemolysis, morphology or antibiotic sensitivity were observed, the mutant strain showed elevated carbon source utilization compared with the control group. Genomic and proteomic analyses showed that 801 genes were upregulated and 825 genes were downregulated. In addition, 167 proteins were overexpressed and 92 proteins were downregulated using a cut-off fold-change value of 1.4 and a p < 0.05. The changed proteins were associated with metabolic functions such as alanine and glutamate metabolism, arginine and proline metabolism, and fatty acid and propanoate metabolism.

Conclusions. E. coli showed alterations at gene and protein levels mainly regarding biochemical metabolism after spaceflight. © 2015 IMSS. Published by Elsevier Inc.

Key Words: Space medicine, Escherichia coli, Proteome, Genome.

Introduction

Escherichia coli is a Gram negative bacterial pathogen that commonly resides in the gastrointestinal tract of humans and animals. Most *E. coli* strains are harmless and also constitute part of the normal flora of the gut (1). However, *E. coli* can cause a variety of diseases such as meningitis, bacteremia, septicemia, dysentery, urinary tract infection, pneumonia and cholangitis when the immune system is damaged. Accumulative studies have demonstrated that the chance of contracting infectious diseases caused by *E. coli* strains increased because the host immunity system could be weakened during spaceflight (2,3). On the other hand, the biological

characteristics of microorganisms including gene expression, virulence, bacterial invasion, antibiotic resistance and bacterial adaptability to environment can be changed after exposure to the space environment (4,5). Previous studies have investigated the effect of simulated microgravity on *E. coli*; however, the ground-based simulation environment is partially different from the real space environment because the latter is more complicated. Therefore, it is necessary to explore the effect of airspace environment on *E. coli*, which could be helpful to space crew members and to public health in general.

Our previous study has reported changes in antibiotic resistance of *E. coli* strain to cefazolin, ampicillin, ceftriaxone, azithromycin and ceftazidime (4,6). However, changes in respect to genome and proteome remained to be elucidated. In the present study we characterized the global changes of *E. coli* after 398 h of spaceflight on the Shenzhou VIII spacecraft by using combined phenotypic, genomic and proteomic analytic approaches.

Address reprint requests to: Xiangqun Fang or Changting Liu, PhD, MD, Nanlou Respiratory Diseases Department, Chinese PLA General Hospital, Beijing 100853, China; Phone/Fax: +86-10-66937592; E-mail: xqfang301@163.com or changtliu@yeah.net

Material and Methods

Acquisition of the Mutant

The ancestral *E. coli* strain (CGMCC 1.2385) was obtained from the Chinese General Microbiological Culture Collection Center (CGMCC). The *E. coli* strain (CGMCC 1.2385), together with 14 other species of microorganisms (4,7–10), were carried in the Shenzhou VIII unmanned spacecraft from November 1–17, 2011 (398 h). The *E. coli* strain was cultured in the special plastic containers that were designed for this study as previously described (7,8). Briefly, another plastic container as a control group was simultaneously placed in an incubator that simulated the temperature exposure of space. When the rocket returned, a ground control named LCT-EC226 and one space strain named LCT-EC67 with distinct phenotypic features from LCT-EC226 were selected for further analysis.

Phenotypic Analysis

Morphological analysis, disk diffusion tests, hemolysis assay, growth curve calculation and biochemical characterization were performed as previously described (7,8).

Genomic and Transcriptomic Analysis

Genome sequencing was conducted by Beijing Genomics Institute (BGI, China). Briefly, the genomic DNA for each bacterium was prepared by conventional phenol-chloroform extraction methods. Two paired-end libraries were generated with average insert sizes of 500 bp and 6 kb following the manufacturer's instructions. The whole genome was sequenced to average depths of 100 and 50 coverage from the two libraries, respectively, with a read length of 90 bp. Low quality reads were filtered using the DynamicTrim and LengthSort Perl scripts within SolexaQA. Short reads were

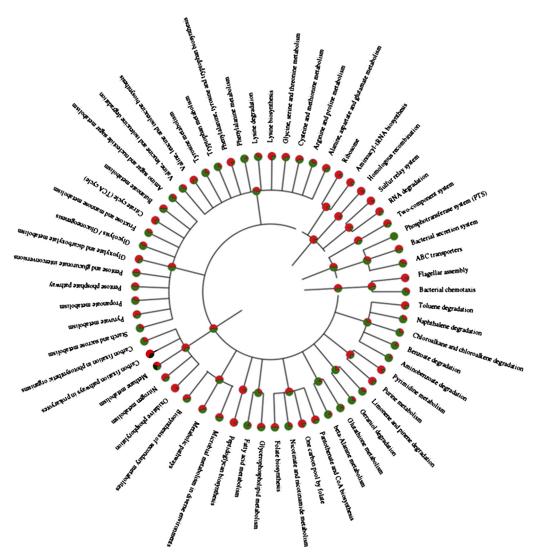


Figure 1. The inner circle represents the first class of KO function classifications, the mediate circle indicates the second class and the outer circle stands for the third class. Red color shows gene expression of LCT-EC67 strain is downregulated in comparison to LCT-EC226 strain and green color indicates upregulation. (A color figure can be found in the online version of this article.)

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