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TThe mutation induced by space conditions in *Escherichia coli*

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INTRODUCTION

Progress has been made in microorganism breeding under space conditions by board ing on recoverable satellite and high altitude balloon in China. To further study the mutagenesis in space, three strains of *E. coli* were put on board the recoverable satellite (JB1-B9611020) launched in October. 1996. After the satellite returned to the earth, the survival and mutation frequencies were determined and the results were discussed as well.

MATERIALS AND METHODS

Bacterial strains

CSH108[1], an arginine autotrophic (Arg) strain, was provided by the *E. coli* Genetic Stock Center, USA (CGSC) and was used to study Arg^+ reversion mutation. Both Arg^- and $LacZ^-$ in CSH108 were caused by amber mutation. The strain A2 and A3 were constructed for $lacI^-$ mutation in this study (the detailed procedure was not described here). A3 was a lacI-qstrain used for the selection of $LacI^-$ mutation after boarding, while A2 is a $lacI^-$ strain serving as a control strain. The properties of flight *E. coli* strains are listed in Table 1.

Table 1 Properties of the boarded E. coli strains

Strain	Genotype
A2	*ara(lac proB)strA/F'lacI* $^-$ *proA*$^+$*B*$^+$
A3	*ara(lac proB)strA/F'lacI*^{q} *pro*$^+$
CSH108	*ara(gpt-lac)gyrA argE*$_{am}$ *proB/F'lacI*$^-$ *Z*$^-$*proA* $^+$*B*$^+$

Boarding methods and space conditions

Since *E. coli* strains usually are hard to survive from space board, soft agar culture of *E. coli* cells was made in this boarding. It was prepared as below: the cells grown on plate were suspended in a small amount of LB liquid medium, and then added melted sterile agar to get soft agar culture (the final concentration of agar was 3.5 μg/L).

In order to study the mutagenesis induced by different factors in space conditions, the boarding samples were divided into three groups and each group included the three strains. When boarding on the satellite Group I was held in small polymethyl methacrylate tubes, Group II was placed in a centrifuge inside DM-11 small biocabin, where oxygen was supplied[2] and the gravity was adjusted to 1g, and Group III was placed in a lead chamber (usually used to store radioisotope) which had 3.5 mm-8.0 mm thick wall and coated with 5 mm layer of hard plastics outside. The lead chamber could block part of t he radiation, but the exact amount of block efficiency was not determined in this study. Part of the ground control bacteria was placed in a dark vessel at room temperature (13 ℃-20 ℃), the other was stored in a freezer (-60 ℃).

The satellite flew for 15 days. The angle of satellite orbit was 63°, apogee was 354 km, perigee was 175km, microgravity was 5×10^{-5} g, density of high energy particle was 136 counts/cm^2 [it was (35.6±6) counts/cm^2 on the earth]. The records show that the biocabin worked regularly in flight, in which the temperature was 17 ℃-26 ℃ and the mean dosage of ionizing radiation was 0.177 mGy/d.

Mutation frequency of the bacteria

The mutation frequency was measured soon after the flight. The procedure was as follows: 0.5 mL of the boarded and control samples were inoculated in 5 mL of LB medium respectively. After 4 h at 37 ℃, 2.5 mL of 50% sterile glycerol was added, and all samples were divided into 1 mL of aliquot, and stored at -20 ℃. During the measurement, an aliquot of the sample was used for bacterial cell counting, and then concentrated sample was spread on screening plates to select mutants. The number of mutants was scored in 48 h-72 h incubation at 37 ℃. The mutation frequency of bacteria was calculated according to the formula: The mutation frequency=mutant cells per mL/total bacterial cells per mL.

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Media

The LB medium, minimal medium, MacConkey medium and Pgal medium were used in experiment as described[1].

Selection of Arg^+ reversion mutants and test of Arg^+ Lac^+ mutants

The concentrated CSH108 sample was spread on minimal plate without arginine. The Arg^+ revertants could grow after incubation. Then the Arg^+ revertants were streaked on minimal plate with lactose as the only carbon source. If some of the revertants could grow up on the medium, they were Arg^+ Lac^+ revertants. These were determined by the method of Miller[1].

Selection of $LacI^-$ mutant

Phenyl-β-D-galactoside (Pgal) is a noninducing sugar that se rves as a substrate for β-galactosidase and can provide a carb on source for growth, but only the constitutive mutants of $LacI^-$, which produce enough β-galactosidase, can form colonies on Pgal[1]. To prove to be $LacI^-$ mutants, the colonies grown on the Pgal plate were streaked on MacConkey plate to compare with A2 strain as the $LacI^-$ control.

RESULTS

The survival of the boarded strains

Before boarding, the cell counting of samples was up to 2×10^9/mL and after flight the survival of each sample was about 3×10^8/mL counted on LB plate, suggesting that the survival of the strains was accomplished as expected in this study.

The Arg^+ reversion frequency of strain CSH 108

After flight the Arg^+ reversion frequencies of CSH108 in the three groups and the ground control were determined. The results are shown in Table 2 (the reversion frequency of each was the mean of seven tests). In fact the Arg^+ reversion frequency of the ground control was the spontaneous reversion frequency. It was worth mentioning that the Arg^+ reversion frequency of Group III was 10 times that of the ground control.

Table 2 The Arg^+ reversion frequency of strain CSH108 in different boarding ways

Sample	Group	Arg^+ reversion frequency($\times10^{-8}$)
Boarding sample	Group I	2.9
	Group II	1.8
	Group III	26.3
Ground control	A(1)	1.2
	B(2)	2.8

(1) Ground control: strain was kept at room temperature; (2) Ground control: strain was stored in freezer (-60℃).

The Lac mutation frequency among Arg^+ revertant

The Lac^+ mutation frequency of CSH108 varied with the groups. As shown in Tables 2 and 3, Group III had not only a high Arg^+ reversion frequency, but also a high Arg^+ Lac^+ frequency. It is suggested that most of the Arg^+ revertan ts were suppresser mutations, which resulted from mutations located in tRNA genes.

Table 3 The occurrence of Lac^+ phenotype among Arg^+ revertants

Sample	Group	Total No. of Arg^+ reversion	No. of Lac^+	Lac^+/Arg^+ (%)
Boarding sample	Group I	60	25	41.7
	Group II	139	52	37.4
	Group III	384	376	97.9
Ground control	A(1)	36	14	38.9
	B(2)	39	27	69.2

A(1) and B(2) are the same with that in Table 2.

The $LacI^-$ mutation in A3 strain

In A3 strain, the survival and $LacI^-$ frequencies are shown in Table 4. The $LacI^-$ frequency in Group II was remarkably higher than that in other groups and it was 67 times that of the ground control. In addition, we also observed that when the boarded strains were plated on Pgalagar at 37℃, Group II formed colonies in 48h, while other groups formed colonies in 72h. From Table 4, it also can be seen that the $LacI^-$ mutation frequency in Group III was 4.4 times that in the ground control. A further test showed that most of the $LacI^-$ mutation in A3 strain could not be suppressed in suppresser strains, therefore they were not amber mutations (The detailed result was not described here).

Table 4 The survival and frequency of $LacI^-$ mutant from A3 strain

Sample	Group	Survival ($\times10^8$)	$LacI^-$ frequency ($\times10^{-8}$)
Boarding sample	Group I	3.6	0.4
	Group II	5.5	240.0
	Group III	3.0	15.8
Ground control	A(1)		3.6

All figures were means of four tests. (1) Ground control: strain was kept at room temperature.

DISCUSSION

Arg^+ revertant

Reversion mutation is a simple and accurate method used to determine the mutation frequency of bacteria[5]. At least two kinds of mutations can reverse the Arg^- (arginine synthesis defective)

phenotype in CSH108: the mutation at $ArgE_{am}$ position and the suppresser mutation. They are both point mutations, but occur in different places. In the revertants with only Arg^+ phenotype, the mutation results from a base substitution at $ArgE_{am}$ position to restore Arg^+ by a sense triplet; in the revertants with Arg^+ Lac^+ phenotype, the mutation occurs in tRNA gene and gets the intergenic repressor by suppresser mutation[1,3]. According to the results shown in Table 3, the revertants of suppreser mutation in Group III covered 97%, while the frequen cies of such mutation were below 70% in other groups, usually about 50% (Table 3).

LacI⁻ mutant

lacI gene encoded repressor for *lacZ* gene. $LacI^+$ bacteria could not grow in Pgal plate unless they were mutated to $LacI^-$ strain. According to the results in Table 4, the $LacI^-$ mutation frequency in Group II was 67 times that of the ground control, and was 4.4 times in Group III that in the ground control. Both mutation and reversion are often used in microorganism genetic experiment[1]. It is convincing to use the markers in this study to investigate the mutagenesis of microorganism in space conditions.

Boarding methods

Three boarding methods were used, and the reversion mutation frequency of Arg^+ and the mutation frequency of $LacI^-$ were measured in this study. The samples of Group II were placed in DM-11 small biocabin, therefore the microgravity had little effect. The main factor affecting the samples was space radiation. In addition, oxygen was supplied in biocabin. It had been reported that in mammalian cell the break incidence of single-stranded DNA in O_2 environment was four times higher than that in no O_2 environment[4], and the occurrence of mutation was closely related with the repair of DNA damage[3,5]. These are probably the reasons why the $LacI^-$ mutation frequency of A3 strain in biocabin was 67 times that of the ground control.

The small lead chamber could block part of the space radiation in flight. The results showed that in lead chamber the Arg^+ reversion frequency of CSH108 was 10 times that of the ground control, and that the $LacI^-$ mutation frequency of A3 strain was 4 times that of the ground control. In this test, the effect of space radiation was decreased by the chamber, the main effective factor should be the microgravity.

The boarded Group I was influenced by microgravity and strong space radiation, but no significant effect on mutation in *E. coli* strains was found. It may be due the interference of the samples located in the satellite, or the antagonis m between different space factors, or some unknown reasons.

It was shown that after flight some *E. coli* strains had high mutation frequencies which varied with boarding conditions. That is to say, in different boarding conditions, there were different space factors that influenced the bacteria mutagenesis, therefore different types and frequencies of mutations were induced. The effects of spaceflight have been increasingly understood[1]. This research indicated that the spaceflight may greatly enhance the mutation frequency of certain genes in microorganism and may provide an effective way for microorganism breeding. But space factors, such as strong radiation, microgravity and so on, which influence the mutation of *E. coli* are complicated. The mutation effect would vary with strain, gene, and even the nucleotide location in DNA. Therefore much work is to be done in understanding the mechanism of space induced breeding. In addition, it is meaningful to take the advantages of quick growth and clear selective markers in *E. coli* strains to develop a high-speed routine method for predicting the space induced efficiency. The method can serve all the purposes of biological investigation.

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