ANALYSIS OF A MICROBIAL BIODIVERSITY AND COMMUNITY STRUCTURE OF THE AIR ASSOCIATED MICROBES IN THE REGENERATIVE ENCLOSED LIFE SUPPORT MODULE SIMULATOR

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1. BACKGROUNDS AND PURPOSES

Regenerative Enclosed Life Support Module Simulator (REMS) is a mockup of the International Space Station (ISS) that USA is playing a central role of construction. In the REMS, the crews performed the same activities as those in ISS, such as daily exercises, cooking, sterilizing and so on. It is known that the immune activity of astronaut depresses severely in the space. Therefore, it is important to control microbes including the opportunistic pathogens for the health maintenance and disease prevention of the crews. The airborne bacteria in the REMS had been detected by the plate count method, which detects only bacteria that can grow in the specific condition. Therefore, overall airborne microbial biodiversity and community structure in those spaces had poorly been understood. Clone library method is known to be one of the most improved methods to detect the microbial biodiversity and the community structure exhaustively and sensitively. The purpose of the study is detecting microbial biodiversity and clarifying the microbial community structure in the REMS using the clone library method.

2. MATERIALS AND METHODS

We collected 9 samples from 9 different spots each month since February, 2005. According to the Colony Forming Unit (CFU)/ Intracellular ATP ratio, which indicate the Gram-positive bacteria / Gram-negative bacteria ratio in the sample, I selected the highest, the lowest and the middle ones from samples of each month. I extracted the total DNA directly from samples and amplified the 16S rDNA fragment (about 1,400 bp) by the PCR. I inserted the PCR products to the vector and transform it to the E-coli and made clone libraries. After the RFLP and sequencing, I identified the air associated microbes and the structure of it.

3. RESULTS

A clone library method detected 23 Gram-positive bacteria and 22 Gram-negative bacteria, including α, β, γ –proteobacteria. The number of the microbes detected by the clone library method is much more than that by the plate count method (12 Gram-positive bacteria and 5 Gram-negative bacteria (all are α-proteobacteria)). Some opportunistic pathogens (Acidovorax, Acinetobacter, Moraxella, Micrococcus, Staphylococcus) were retrieved. And, the percentage of the Gram-negative bacterium in clone library was more than 75%, which is bigger than that detected by plate count methods. The main species in the community structure were different depending on the sampling month and place.

4. Discussion

Clone library method revealed that diverse microbes were existed in the REMS. This method was more sensitive so that we could detect opportunistic pathogen which could not be detected by the plate count method. This study indicates that these opportunistic pathogens can be detected in the ISS whose inside condition is similar but more difficult to control than that in REMS. From now, we need to examine the microbial biodiversity in the ISS carefully by the clone library method, and reveal the ecology of the opportunistic pathogens which could not be detected so far. In addition to that, we also need to develop a hygienic system which enables us to estimate the affection of the opportunistic pathogen on crews, to detect the increasing of them and remove quickly.