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Microbial community structure and succession of airborne microbes in closed artificial ecosystem

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ABSTRACT

Microbes play a significant role in achieving substance circulation and regeneration in closed artificial ecosystems (CAES) such as bioregenerative life support system (BLSS), greenhouses, and aquaria. In order to understand the pattern of microbial development inside such closed systems, a ground-based closed comprehensive BLSS experimental system named "Lunar Palace 1 (LP1)" was established and a 105-day manned BLSS experiment was carried out in LP1 by our team. During this closed experiment, airborne microbes in the closed cabins of LP1 were sampled and the development and succession law of microorganisms were analyzed using both plate cultivation and molecular biology methods. The results indicated that the dominant bacteria in the air of LP1 were *Cupriavidus*, *Afipia*, *Delftia*, *Cyanobacteria* and *Enterococcus* and the dominant fungi were *Penicillium*, *Alternaria*, *Aspergillus* and *Cochliobolus*, which included not only ubiquitous environmental microorganisms but also opportunistic pathogens. And the succession of dominant microbes' composition also provided an important experience for solving the problem of microbial contamination in CAES and set a solid foundation for establishing more targeted microbial prevention and control measures in long-term multi-crew closed BLSS experiments in the future.

1. Introduction

Closed artificial ecosystems (CAES) are closed ecosystems designed and controlled by humans ranging from agricultural systems, bioregenerative life support systems (BLSS) to microcosms, and aquaria, which may be widely useful in practical or research applications (DeAngelis, 2003; Pechurkin and Shirobokova, 2001). BLSS, as one typical CAES, consists of plants, animals and microorganisms and provides the crewmembers with a life support environment similar to the ecosystem on the Earth (Liu et al., 2012). For such closed ecosystems like BLSS, grasping the law of microbial development is particularly critical for the control of microbial contamination in the system, which is the key to maintain normal operation of the system.

Since the 1970s, ground-based BLSS test systems have been built, simulated BLSS experiments have been launched, and microorganisms in the BLSS system have been studied. In the early 1970s, the Russian experts have begun to study plant microorganisms in BIOS-3 system. Tirranen (2008) studied the microbial community structure of plant root zone, radish phyllosphere, seeds and other parts of plants, measured the total colony number of anaerobic and aerobic bacteria, fungal spores, plant pathogenic bacteria, yeast and actinomycetes, and revealed the microbial community formation process on plants and its determinant factors. Biosphere-2 introduced more than 3800 species of organisms, including more than 1000 species of microorganisms (Nelson et al., 2008). However, due to the rapid reproduction of soil microorganisms in the system, the balance between the respiration of experimenters and soil and the photosynthesis of plants within the system was disturbed, hence the closed test could not be continued in the end. Japanese Institute of Environmental Sciences established a Closed Ecology Experiment Facilities (CEEF) to study the migration and transformation of radioactive substances in the ecosystem. In CEEF system, microbial waste disposal unit played an important role as the basic design of system components (Nitta, 1999). In addition, in Micro-Ecological Life Support System Alternative (MELiSSA) system officially launched by Canada European Space Agency (ESA), Belgium and Spain, the microbial reactors constitute

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an important part in the waste recycling unit, which mainly consists of waste degradation room made up of thermophilic anaerobic bacteria reactor, photosynthesis heterotrophic food production room made up of photosynthesis heterotrophic bacteria *Rhodospirillium rubrum* reactor, and nitrification room made up of nitrite bacteria and nitrification bacteria reactor (Hendrickx et al., 2006). Microbes are important producers or decomposers in BLSS. However, if the functional microbes in the closed system or the complex microbes that the system carries itself could not be effectively controlled, microorganisms can possibly be a major threat to other organisms in the system and the balance of the system itself.

Microbes cause harm to closed system mainly in two aspects. For one thing, it can threaten the health of organisms including the crew, plants and animals; for another, it can give rise to material corrosion within the cabin, affect the normal operation of the system, and even lead to failure of the entire closed BLSS experiment. Therefore, effective microbial control procedures should be carried out in the experiment. Moreover, effective microbial preventive measures should be taken before the closed experiment. In order to establish a more accurate and efficient microorganism control system, it is necessary to understand the main sources and composition of microbes in closed experiment and the law of their development and succession during the experiment.

"Lunar Palace 1 (LP1)" is a ground-based comprehensive experimental CAES that our team established to research the key unit technologies of BLSS. In this closed system, we carried out a 105-day BLSS experiment with multi-crew involved from 3rd February, 2014 to 20th May, 2014. In order to provide support for the effective control of microorganisms in such a closed system, the growth pattern of microorganisms within the system should be mastered first (Liu et al., 2012). Therefore during the 105-day closed experiment in LP1, airborne microbes in the experimental cabins were sampled, the dominant bacteria and fungi were identified using both plate cultivation and molecular biology methods, and the development and succession law of microorganisms were analyzed and discussed accordingly.

2. Materials and methods

2.1. Sampled points

LP1 is composed of a plant cabin with 2 separate rooms and a comprehensive cabin including 4 bed rooms, a waste treatment room, a bathroom and a living room. In this study, air samples were collected from 7 sampling points as shown in Fig. 1, including 2 sampling points in plant cultivation room, 2 in bedrooms (inhabited), 1 in waste treatment room, 1 in bathroom and 1 in living room.

The color blue marks sample points where the air sampler was placed. No. 1 sampling point: plant cabin-room 1; No. 2 sampling

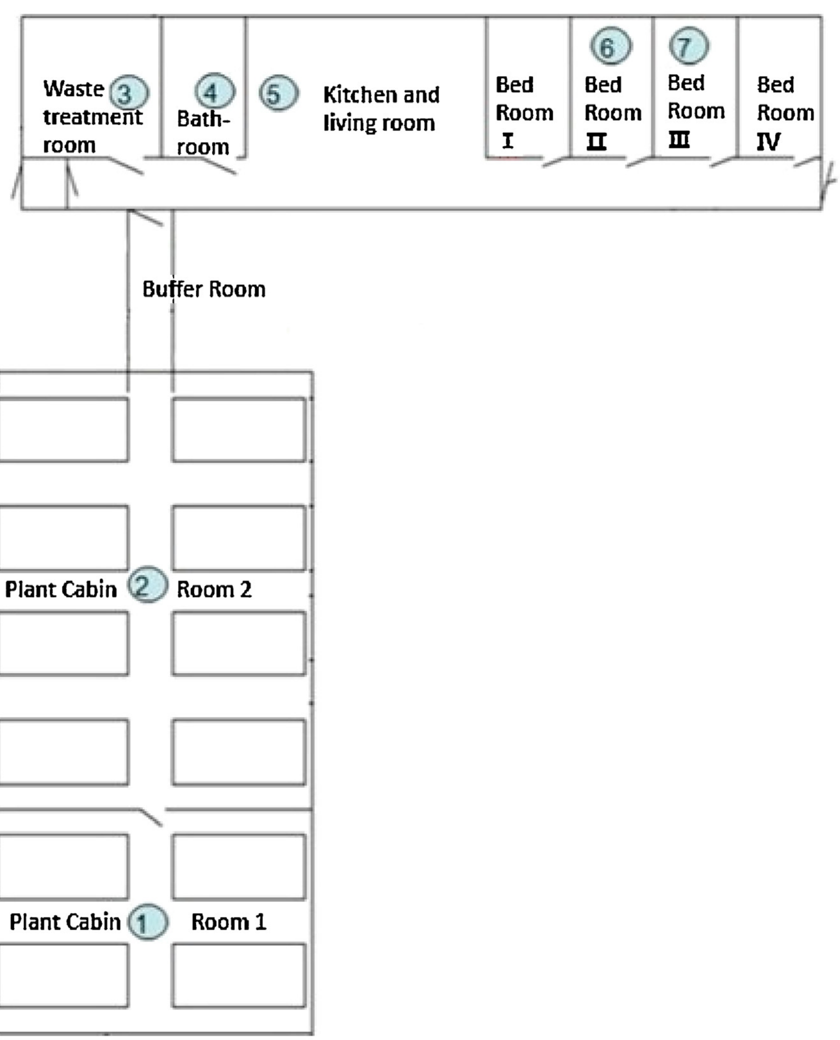

Fig. 1. Layout of "Lunar Palace 1".