Microbial ecology at the International Space Station
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Chapter 1 General introduction

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Microbial hygiene is an important aspect of our modern society and the monitoring and control of microbial contamination plays a pivotal role in clinical settings, the veterinary field, the food industry, potable water distribution systems or air-conditioning systems in modern buildings, but also in advanced life support systems such as manned spacecraft. Moreover, concerns about microbial outbreaks such as anthrax in relation to biological warfare and terrorism or more recently the enterohaemorrhagic \textit{Escherichia coli} (EHEC) have added to the need for the development of rapid and easy to use on-site detection methods to assure microbial hygiene.

The \textit{International Space Station} (ISS) is an orbital living and working environment with a controlled atmosphere that forms a unique microbial ecological niche\cite{13} which encompasses several spaceflight related parameters\cite{4} such as microgravity, radiation and semi-closed environment (Fig. 1). Since the ISS started out as a sterile environment at the beginning of its construction in 1998, virtually any of the surfaces of its interior has been colonised by microbes. Microbial colonisation may originate from various sources of which the crew themselves are thought to be the main source of contamination, distribution and means of nutrition\cite{15,18}. The complex microbial ecosystem that is accommodated by the human body, the human microbiota, plays a crucial role in health and disease\cite{2,19}. Most microbes are harmless or beneficial to human health and some will likely play an important role in making long-term space habitation feasible, such as in air and water purification or solid waste remediation.

\textbf{Health and safety issues of microbiological origin on board of the International Space Station}

In contrast, hazardous microbes such as (potential) pathogens and so-called ‘technophiles’\cite{16,23} may form a serious problem in spacecraft such as the ISS\cite{18}. They not only pose a threat to the health of the crew, but also to the integrity of technical equipment and materials of a spacecraft. Technophiles are defined as microbes that can colonise and deteriorate practically any technical material such as glass, metal, polymers or a combination thereof and may therefore cause damage to the equipment, which can lead to severe technical problems\cite{16,18}. Furthermore, for a range of microbial species typical growth kinetics and physiology characteristics under spaceflight conditions have been reported, such as increased proliferation\cite{6,8,9,13,14}, enhanced virulence\cite{10,14,24}, increased antibiotic resistance\cite{10,13,14,22}, increased mutation rate\cite{21,22} and differential gene expression\cite{4,24}. In addition, the immune system of man tends to become negatively affected\cite{20}. These factors taken together pose serious risks to the health and safety of the crew of a space station, especially on long-duration missions\cite{10,22}. It is therefore essential to gain insights in the above processes and to characterise the microbial contamination on board of the ISS\cite{1,15,18}. These issues were addressed by a consortium of European scientists and companies in this
Figure 1. Overview of the main structural elements of the ISS (at assembly complete, as of February 2009). Source: http://www.nasa.gov/mission_pages/station/news/index.html.
“Microgravity Applications Programme” of the European Space Agency (ESA-MAP) and the ‘SAMPLE’ experiments.23.

Rapid and easy to use molecular methods for the on-site detection and quantification of microbes

Especially for remote locations such as the ISS or comparable environments on Earth, rapid and easy to use methods that can be used on-site without the need for specialist expertise are invaluable for the detection and quantification of microbes. For the use on-site, in particular on board of a spacecraft, microbial detection methods are preferred that take up as little volume and weight as possible during storage and transport due to high transportation costs and limited room for storage. In addition, the methods should be safe to perform and the use of toxic chemicals and materials should be minimised.

Conventional culturing techniques however do not meet these criteria. Furthermore, the culturing of hazardous microbes is not always desirable, especially on a remote location such as a space station where medical facilities are absent. Moreover, the detection of many microbes by culturing techniques is hampered as they are difficult to culture or uncultivable. Molecular methods overcome many of the problems encountered by conventional culturing techniques and are not limited by cultivation artefacts. Accordingly, molecular methods have increasingly been used for the detection and quantification of hazardous microbes. The development of rapid and easy to use molecular methods for the detection and quantification of hazardous microbes for application in manned spacecraft is therefore desirable and may also be valuable for applications on Earth. Quantitative real-time polymerase chain reaction (qrtPCR) is such a molecular method for the rapid detection and quantification of nucleic acid sequences. The method has a high specificity with a detection sensitivity of a few molecules per reaction and the potential to be automated and miniaturised for use on-site by non-specialists.

Outline and scope of the thesis

One of the aims of the studies described in this thesis was to study which effect spaceflight conditions have on the normal microbiota of the crew. Another aim was to map the dynamics of microbial contamination on board of the ISS and gain insights into which type of locations are most susceptible to microbial growth. A final aim was to develop rapid and easy molecular methods for the on-site detection and quantification of hazardous microbes and to apply these methods during the implementation of the aims mentioned above.

Rapid on-site quantitative detection of bacteria by molecular methods such as qrtPCR requires rapid and easy to use sampling and sample processing methods that efficiently recover bacterial DNA suitable for amplification. Accordingly, the efficiency of the used
methods as a whole, including the preceding steps of sampling and sample processing, needs to be assessed in order to give a reliable estimate of the quantity of bacteria present.

In Chapter 2, the first step preceding the molecular method of qrtPCR, sampling, is evaluated for the purpose of rapid on-site quantitative detection of bacteria. In addition, the efficiency to recover DNA from bacterial cells of the model organism Escherichia coli that were pre-applied and dried up on glass surfaces was determined for four selected swab systems with qrtPCR.

The second step preceding the method of qrtPCR, that of sample processing, is evaluated in Chapter 3. Sample processing for use with qrtPCR mostly involves cell lysis and the subsequent recovery of DNA free of amplification inhibitors. The recovery of DNA from defined numbers of bacterial cells that were subjected to three different DNA extraction methods was measured using qrtPCR for E. coli and Staphylococcus aureus.

A general assumption that is made, is that during DNA extraction, cells used for the generation of standards for quantitative molecular methods such as qrtPCR or cell spiking in experimental settings release DNA with the same efficiency as the cells that are to be sampled. Chapter 4 addresses this assumption and gives new insights to this issue.

To apply the developed tools and to get a better understanding in the above mentioned processes, a series of spaceflight experiments were performed, the ‘SAMPLE’ experiments. During several missions to the ISS in 2004, 2005, 2006 and 2007, samples were taken from the microbiota of three cosmonauts and from thirty-five distinct locations of the interior of the Russian Zvezda Service Module (DOS-8) of the ISS (Fig. 1).

Knowledge on human microbiota changes under spaceflight conditions is very scarce and has mainly been limited to comparisons of pre- and post-flight data due to the significant constraints of spaceflight experiments. Chapter 5 describes the findings of in-flight changes of the bacterial microbiota of three cosmonauts during short missions to the ISS by using molecular methods without prior culturing, such as qrtPCR, Denaturing Gradient Gel Electrophoresis (DGGE) and 16S rDNA sequencing, as well as conventional culturing techniques.

Chapter 6 describes the evaluation of microbial contamination of the interior of the Zvezda Module. The microbial composition of the samples taken from the Zvezda Module was determined by the same methods as described in Chapter 5, with the addition of clone libraries. The sampling locations were chosen in such a way that they likely represented presumed distinct microbial niches, such as computers, human contact areas, walls, toilet cabin or technical equipment.

In the frame of monitoring and control of microbial contamination, Chapter 7 describes the isolation and phylogenetic affiliation of an unidentifiable Gram-positive, motile, rod-shaped, endospore-forming facultative aerobic bacterial strain found in a sample taken from the interior of the Zvezda Module.

Chapter 8 describes the challenge posed in correctly identifying virulent Bacillus anthracis, the etiologic agent of the disease anthrax, in environmental samples of mixed
microbial communities. This issue is illustrated by a case-study of the troublesome phylogenetic affiliation of isolates of the Bacillus cereus group isolated from the Zvezda Module, by using both culturing techniques as well as molecular methods.

References


