Mold Species in Dust from the International Space Station Identified and Quantified by Mold Specific Quantitative PCR

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Abstract

Dust was collected over a period of several weeks in 2007 from HEPA filters in the U.S. Laboratory Module of the International Space Station (ISS). The dust was returned on the Space Shuttle Atlantis, mixed, sieved, and the DNA was extracted. Using a DNA-based method called mold specific quantitative PCR (MSQPCR), 39 molds were measured in the dust. Potential opportunistic pathogens *Aspergillus flavus* and *A. niger* and potential moderate toxin producers *Penicillium chrysogenum* and *P. brevicompactum* were noteworthy. No cells of the potential opportunistic pathogens *A. fumigatus*, *A. terreus*, *Fusarium solani* or *Candida albicans* were detected.

Keywords: International Space Station, mold specific quantitative PCR, *Aspergillus*

1. Introduction

Since human space exploration began, microbes have traveled with us and are ubiquitous throughout the spacecraft. Previous studies have demonstrated that bacteria, including potential pathogens, were commonly isolated in the air, water, and on surfaces aboard the Mir Space Station [12] and the International Space Station (ISS) [1,6]. Biofilms were found in the water distribution lines on the Space Shuttle Discovery [5].

Another important group of microbes are the fungi or molds. Molds like *Penicillium chrysogenum* were measured on the Mir Space Station from the beginning of its on-orbit service [19]. Six “space fungi” including *P. chrysogenum* and *Aspergillus versicolor* were identified by morphology and sequence analysis and were shown to be amplifying on the Mir [7]. The authors concluded that “the environment aboard the space station Mir allows the growth (of mold) as (is) true in residential areas on earth”. A six year survey of the ISS showed the concentration of molds ranged from a high of less than 4.4 x 10^1 CFU/m^3 air to 4.3 x 10^4 CFU per 100 cm^2 of surfaces [11]. However, these analyses were all based on culturing the molds. It is important that timely information about the concentration of molds in the ISS is available, since molds present a threat to the integrity of the health of the cosmonauts/astronauts manning the ISS and the components of the ISS itself [11].

Potential health threats from molds include infections, toxicity and allergic reactions such as asthma. Although most molds are not a threat to humans with a healthy immune system, numerous studies have shown that the immune system of many astronauts were affected as a result of space flight and habitation [3,4,8,9,13,15,16]. Most parameters of the immune system were altered, usually decreased, resulting from various factors associated with space flight. However, mold infections usually occur only in the severely immuno-compromised. Although no known mold related health effects have occurred among the astronauts as a result of their time aboard the ISS, monitoring of these mold species is important for evaluating the threat.

Mold growth is also potentially problematic for the integrity and operation of the ISS itself. Molds are saprophytic and therefore, to grow and multiply, they must breakdown organic substrates, which could be parts of the ISS (Figure 1).

In this study, a rapid (<2 hour), DNA-based method of mold analysis called mold specific quantitative PCR (MSQPCR) was used to identify and quantify mold species found in the dust collected aboard the ISS. The focus was the 36 species common to environments on earth, as described by the Environmental Relative Moldiness Index (ERMI) [17], and three additional opportunistic pathogens.

2. Materials and methods

A vacuum bag dust sample was collected from HEPA filters in the U.S. Laboratory module of the ISS. HEPA filters are strategically installed at selected "returns" located throughout the US segment of the International Space Station. Vacuuming of the HEPA filters is a part of the nominal housekeeping activities on-board the ISS. The dust was returned on the Space Shuttle Atlantis in 2007. The dust was thoroughly mixed and then sieved through a 300 micron pore size nylon mesh (Gilson Company, Inc. Lewis Center, OH). The DNA was extracted and purified from the samples using the GeneRite DNA-EZ™ kit, as directed (KC101-04C-50; GeneRite, Kendal Park, NJ). Two replicate analyses of 5.0 ± 0.1 mg dust each from the sample were performed [17]. All primer and probe sequences, as well as known species comprising any assay cluster were published at the EPA website: http://www.epa.gov/microbes/moldtech.htm. Negative as well as positive controls for each assay were tested along with the dust analysis.

3. Results and discussion

The results of the MSQPCR analysis of the dust from the ISS are shown in Table 1. The most common species measured was *P. chrysogenum* (average 350 cell equivalents per mg dust) followed by *Aureobasidium pullulans* (average 96 cell equivalents per mg dust). Of the 36 species in the standard ERMI analysis, 17 were detected at some concentration. Three additional opportunistic pathogens, *A. terreus*, *Fusarium solani*, and *Candida albicans*, were not detected in the dust (data not given).

Common species causing aspergillosis that were detected included, *A. flavus* and *A. niger* (average 27 and 92 cell equivalents per mg dust, respectively). Additional, occasional pathogens and toxin producing species measured on the ISS were *P. brevicompactum* and *A. sydowii* (average 32 and 16 cell equivalents per mg dust, respectively). *Stachybotrys chartarum* was not detected in the ISS dust.

This ISS dust sample contained the opportunistic pathogens *A. flavus* and *A. niger* at concentrations higher than the geometric mean measured in homes across the U.S. [17]. Just as important, the tests demonstrated that other opportunistic pathogens including, *A. fumigatus, A. terreus, F. solani and C. albicans* were not present (at least at this time and in this sample).

In addition to infections, many molds produce mycotoxins and allergens which can affect the immune system and other organs. For example *A. flavus* is the source of aflatoxin, a known carcinogen. Other molds quantified by MSQPCR on the ISS included *P. chrysogenum* and *P. brevicompactum* which produce toxins that can cause inflammatory and cytotoxic responses [14]. These toxins or allergens may be active, even if the cells that possess them are not viable.

Historically, the ISS environment was monitored by both the U.S. and Russia in their respective modules by periodically collecting samples from the air and various surfaces [12]. The air and surface samples collected by astronauts in the U.S. segment were incubated on-board the ISS and colony counts recorded by the crew. This approach gave the crew an estimate of the number of viable microbes relatively quickly (depending on microbial growth rate). However, for identification of the microbes, the cultures were returned to earth. Typically Russian cosmonauts collected air samples and the crew recorded the number of bacterial and mold colonies after incubation [10]. However, surface samples were collected and stabilized for return to earth, where the microbes were cultured, identified and quantified.

Novikova et al. [11] described two fundamental limitations in these methods used to monitor microorganisms on the ISS: the time gap between sampling and results and the reliance on culture-based analyses. The DNA-based method applied in this study can solve both of these problems. Although, in this case, the dust sample was returned to earth for analysis, a number of companies are developing closed instrumentation platforms, e.g. the IQuum (see www.iquum.com) that would make this analysis available on a nearly real-time basis and could potentially be performed on the ISS.

It would also be desirable to know which mold cells are viable. New methods for discriminating viable and non-viable mold cells, in conjunction with MSQPCR, are becoming available [18]. The results are specific, quantitative and available in about 2 hours. In most cases, the MSQPCR assays are sensitive down to a single cell [2].

Great effort is made to prevent the introduction of molds and bacteria into the ISS. For example, the ISS was designed to prevent surfaces from collecting condensate and to keep the relative humidity less than 70%. HEPA filter are used in the air reclamation and distribution system.

In addition, all modules launched to join and expand the ISS are assembled in clean rooms, and exposed surfaces are disinfected with hydrogen peroxide (3-6%). Hardware is randomly sampled to ensure compliance with standards. Prior to launch, module surfaces are sampled with swabs and/or contact plates to ensure compliance with stringent acceptability limits, i.e. 100 colony forming units (cfu) / 100 cm^2 for molds and 10,000 cfu / 100 cm^2 for bacteria. The air in the module is also monitored using a small portable air sampler. Acceptability limits are 100 cfu / m^3 of air for molds and 1000 cfu / m^3 for bacteria.

During the flight phase, the air and surfaces are monitored to ensure compliance with acceptability limits. Routine housekeeping, including wiping surfaces is conducted weekly by the crew. Any monitoring that detects levels of bacteria or molds above the acceptability limits prompts a clean-up by the crew using a quaternary ammonium disinfectant. The HEPA filters are protected by a grid and these are routinely vacuumed to remove accumulated materials. However, molds and other microorganisms can still enter the ISS with the crew and various materials carried onto the Shuttle or Russian Soyuz.

4. Conclusions and perspective

It is important to emphasize that there have been no known mold infections or other health effects from molds as a result of living aboard the ISS. Of course, this is just one sample and many more samples would be needed to fully describe the mold conditions of the ISS. Novikova et al., [11] opined “Continuous environmental monitoring during the lifetime of the ISS is of paramount importance.” The use of MSQPCR may be one method to reach this goal.

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Notice

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Table 1. Mean and standard deviation (STD) of the replicate analyses of the concentration of each mold species expressed as cell equivalents (CE) per mg in vacuum bag dust from International Space Station. ND = not detected. NA = not applicable.

	Mean	**STD**
Mold Species	**CE./mg**	**CE./mg**
Group 1		
Aspergillus flavus	27	6
Aspergillus fumigatus	ND	NA
Aspergillus niger	92	68
Aspergillus ochraceus	ND	NA
Aspergillus penicillioides	3	2
Aspergillus restrictus	ND	NA
Aspergillus sclerotiorum	ND	NA
Aspergillus sydowii	17	19
Aspergillus unquis	ND	NA
Aspergillus versicolor	ND	NA
Aureobasidium pullulans	96	120
Chaetomium globosum	3	2
Cladosporium sphaerospermum	1	0.7
Eurotium Group	8.0	6
Paecilomyces variotii	ND	NA
Penicillium brevicompactum	32	18
Penicillium corylophilum	4	3
Penicillium crustosum Group	10	7
Penicillium purpurogenum	3	2
Penicillium spinulosum	ND	NA
Penicillium variabile	ND	NA
Scopulariopsis brevicaulis	1	0.7
Scopulariopsis chartarum	ND	NA
Stachybotrys chartarum	ND	NA
Trichoderma viride	ND	NA
Wallemia sebi	3	2
Group 2		
Acremonium strictum	ND	NA
Alternaria alternata	4	2
Aspergillus ustus	ND	NA
Cladosporium cladosporioides 1	17	4

Cladosporium cladosporioides 2	ND	NA
Cladosporium herbarum	ND	NA
Epicoccum nigrum	ND	NA
Mucor Group	ND	NA
Penicillium chrysogenum 2	350	156
Rhizopus stolonifer	ND	NA

Figure 1. Mold stained panels in the ISS hygiene area resulting from mold growth following contact with wet towels. Approximate area affected is 2 m^2.