Fungi and Mycotoxins in Space—A Review

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Abstract

Fungi are not only present on Earth but colonize spacecraft and space stations as well. This review provides an extensive overview of the large and diverse group of fungal species that have been found in space, as well as those corresponding detection methods used and the existing and potential future prevention and control strategies. Many of the identified fungal species in space, such as Aspergillus flavus and Alternaria sp., are mycotoxigenic; thus, they are potential mycotoxin producers. This indicates that, although the fungal load in space stations tends to be non-alarming, the effects should not be underestimated, since the effect of the space environment on mycotoxin production should be sufficiently studied as well. However, research focused on mycotoxin production under conditions found on space stations is essentially nonexistent, since these kinds of spaceflight experiments are rare. Consequently, it is recommended that detection and monitoring systems for fungi and mycotoxins in space are at some point prioritized such that investigations into the impact of the space environment on mycotoxin production is addressed. Key Words: Fungi—Mycotoxins—Space—Biosafety monitoring—International Space Station—Mir. Astrobiology 19, xxx–xxx.

Contents

(1) Health Risks Related to Fungal and Mycotoxin Presence in Space
(2) Survival, Growth, and Adaptation of Fungi in Space
(3) Fungal Species Detected in Russian and International Space Stations
(4) Sources and Prevention of Fungal Contamination in Space Stations
(5) Monitoring Fungi and Mycotoxins in Space: Application of Various Detection Methods
(6) Strategies to Control Fungal and Mycotoxin Exposure in Space: Limits and Decontamination
(7) Recommendations for Future Research and Development
(8) Conclusions

1. Health Risks Related to Fungal and Mycotoxin Presence in Space

Fungi are not only present on Earth but also colonize spacecraft and space stations and are able to adapt to these extreme conditions (Dadachova and Casadevall, 2008; Yamazaki et al., 2012; Yamaguchi et al., 2014). Although most of these fungi typically do not pose a direct infection threat to humans, in a space cabin they can display adverse effects on crew members’ health and the integrity of the spacecraft (Novikova et al., 2006). In general, on Earth, fungal infections mainly occur in severely immune-compromised humans and people who frequently encounter stress conditions (Vesper et al., 2008). However, it is known that astronauts have a compromised immunity due to the prolonged stress of spaceflight; thus, fungi could cause opportunistic infections in space travelers (Yamazaki et al., 2012). Additionally, many fungi are able to produce mycotoxins and allergens that can remain present in such closed space environments, even if the fungal cells are not viable (Vesper et al., 2008). Mycotoxins are important contaminants that can be produced by various fungal species as

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secondary metabolites. Several species of *Aspergillus, Fusarium, Penicillium,* and *Alternaria* fungi are known myco-
toxin producers. Although these toxins are mostly present in very low concentrations (ppb to ppt range), they can cause acute and chronic toxic effects in humans and animals that range from nausea, diarrhea, gastrointestinal problems, ne-
phropathies, and hyperestrogenism to immunotoxicity and carcinogenicity (Bennett and Klich, 2003). The actual impact of fungal species and contaminants such as mycotoxins upon astronauts’ health obviously depends on many factors in-
cluding the extent to which the contaminant actually comes in contact with the skin, airway, or bloodstream and the sus-
ceptibility and current health state of crew members (Maule et al., 2009). Additionally, growth and metabolite production
of fungi on humans, materials, and equipment inside stations are dependent on factors such as specific atmospheric fluid condensates and chemical or anthropogenic contaminants (e.g., human metabolic products) (Klintworth et al., 1999).
Sugita et al. (2016), for example, demonstrated temporal changes in the skin fungal microbiota of International Space Station (ISS) astronauts. During a stay at the ISS, fungal diversity decreased, whereas colonization by *Malassezia* species (human resident yeast) increased, which suggests that certain commensal fungi indeed can proliferate in a closed environment such as space stations (Sugita et al., 2016).

In our view, the main predicted contamination routes by
which astronauts may come in contact with fungi and my-
cotoxins is through contaminated air and surfaces. However, another potential contamination route and corresponding health risks are related to innovations such as regenerative life-support processes to provide, among other things, water and food on long-term space missions (Walker and Granjou, 2017). This might replace, for example, currently supplied and consumed sterilized foodstuffs. To safeguard astronaut health, the potential accumulation of mycotoxins, in particular, within these new systems should be investigated.

Because of the potential health effects related to myco-
toxin exposure, tolerable daily/weekly intake (TDI/TWI)
levels have been established and regulated for various tox-
in. In the event these levels are exceeded, the health of an individual is endangered. To our knowledge, mycotoxins are not measured or controlled in spacecraft and space stations. To protect astronaut health, especially during long-duration missions, it is thus important to monitor not only fungi but also mycotoxin levels on board spacecraft and to define remediation strategies for use when needed.

### 2. Survival, Growth, and Adaptation of Fungi in Space

Several research articles have addressed the potential survival of individual fungal strains in space and/or the in-
fluence of space conditions on the physiology of these or-
ganisms. Gomoiu et al. (2016) reported results concerning the viability of dried fungal spores present in enclosed biocounters after exposure to microgravity and cosmic radiation and during short-term (14 days) and long-term (5 months) missions on board the ISS. These tests included *Aspergillus niger, Cladosporium herbarum, Ulocladium chartarum,* and *Basidiospora halophila,* all of which are considered to be potentially present on the ISS (Gomoiu et al., 2016). Interestingly, *A. niger* and *U. chartarum* spores showed a high viability even after long-term exposure, whereas a reduction in viability was observed for the other strains (Gomoiu et al., 2016). Consequently, the effect of exposure on viability seems to be dependent on the fungal strain. Other studies have confirmed the presence of intact and metabolically active lichenized fungal cells after long-
term exposure (18 months) of *Cryomyces* and *Xanthoria* species to simulated martian conditions aboard the ISS (Brandt et al., 2015; Onofri et al., 2015).

In space, fungi can use the structural materials of the spacecraft as a source of nutrients for growth. Polymeric materials can be degraded since additives and plasticizers present in these matrices are used as carbon sources by fungi (Gu, 2007). Polymide deterioration by *Aspergillus versicolor,* *Cladosporium cladosporioides,* and *Chaetomium* sp. was described in the work of Gu (2007). *Paecilomyces* and *Cladosporium* fungi were cultured from two synthetic polymers within Apollo mission spacesuits and have been found to be capable of degrading the polymers (Breuker et al., 2003). Their growth and survival on suit materials are assumed to be a contributing factor to the deterioration of the interior layers of Apollo spacesuits (Breuker et al., 2003).

Many fungi living on space stations have been found to be pigmented or melanized (Dadachova and Casadevall, 2008). In the previous decade, the discovery of these melanized fungi in high-radiation environments such as space stations raised the possibility that melanins function as energy-
harvesting pigments and could be useful in such irradiated conditions (Dadachova and Casadevall, 2008). However, more recent studies ascribed the protective role of melanins to their ability to absorb electromagnetic radiation and convert the energy into heat or free electrons that remain present in the pigment or are conducted (Pacelli et al., 2017; Selbmann et al., 2018). Consequently, it is possible that melanin absorbs harmful space radiation and thus protect fungi from DNA and cell damage, thereby functioning as radioprotectors (Pacelli et al., 2017; Selbmann et al., 2018).

It has also been considered possible that spacecraft fungi alter their properties and characteristics, which potentially could make them become dangerous for space and Earth inhabitants (Dadachova and Casadevall, 2008). For example, a 7-month exposure of *Aspergillus versicolor* and *Penicillium expansum* to conditions outside the spacecraft revealed many morphological changes such as a significant increase of the polysaccharide capsule and melanin layer in *P. expansum* (Dadachova and Casadevall, 2008). To further investigate how the space environment may affect and change the characteristics of pathogenic fungi, strains of *A. niger* and *Candida albicans* were subjected to simulated microgravity (Yamazaki et al., 2012). No differences in morphology, growth capability, asexual reproductive capa-
bility, or susceptibility to antifungal agents were observed compared to strains kept under Earth gravity (Yamazaki et al., 2012). Sathishkumar et al. (2014) also investigated phenotypic and genotypic changes in *A. niger* and *Peni-
cillium chrysogenum* under modeled microgravity. Similar to what is stated above, no significant stress influence was found on germination, growth, and cell wall integrity (Sa-
thishkumar et al., 2014). Furthermore, investigation of the morphological characteristics and antifungal susceptibilities of *Penicillium, Aspergillus,* and *Cladosporium* species re-
vealed no significant difference from fungal strains on Earth (Satoh et al., 2016). Additionally, a more recent study stated
that *in vitro* growth characteristics, secondary metabolite production, and susceptibility to chemical stresses showed no clear differences between the ISS and clinical strains of *A. fumigatus* (Knox et al., 2016). However, the ISS strains established a significantly higher lethality in a neutrophil-deficient larval zebrafish model compared to clinical strains (Knox et al., 2016).

The results presented above confirm that the environmental conditions on board spacecraft allow growth of potentially pathogenic fungi, which could contaminate spacecraft with allergenic or toxic metabolites such as mycotoxins and cause opportunistic infections, allergies, and intoxication in space, as occurs on Earth (Yamazaki et al., 2012).

### 3. Fungal Species Detected in Russian and International Space Stations

A large effort has been made to identify bacterial and fungal species present in space stations, and investigation of dust particles from the ISS and spacecraft assembly facilities has indicated that cultivable fungi are less present than bacteria (Checinska et al., 2015). Prelift fungal counts tend to be low in the US space shuttle program, and bacteria were typically more critical than fungi for short-term missions (days/weeks). However, it has also been stated that this likely will change to a predominance of fungi in long-term closed space stations (Klintworth et al., 1999). An overview is provided of the fungi that were identified in space stations such as Mir and the ISS.

Fungal contamination was investigated in Russian space stations. On interior surfaces of the Russian Salyut 6, *Aspergillus spp.*, *Penicillium spp.*, and *Fusarium sp.* were found, indicating the presence of various fungi that have the potential to produce mycotoxins and thus form a potential threat to human health (Gu, 2007). Additionally, several of these species have not only been detected on materials of the interior, equipment, and hardware during Russian Salyut missions but also on Mir missions (Klintworth et al., 1999). Many isolates could be distinguished on surfaces aboard Russian orbital stations in different levels and were identified as species/strains that are predicted to produce mycotoxins, including *Aspergillus tamarii* (<10⁵ CFU/100 cm²; aflatoxin [AF]), *Fusarium species* (<10² CFU/100 cm²; fumonisins and triketocenes), *Aspergillus clavatus* (10² to 10³ CFU/100 cm²; patulin), *Penicillium viridicatum* (10² to 10³ CFU/100 cm²; ochratoxin A [OTA]), *Alternaria alternata* (10⁵ to 10⁶ CFU/100 cm²; e.g., alternariol, tenuazonic acid), *Aspergillus niger* (10⁵ to 10⁶ CFU/100 cm²; OTA) and *Aspergillus versicolor* (10⁴ to 10⁵ CFU/100 cm²; sterigmatocystin) (Klintworth et al., 1999). Additionally, a number of detected species are reported to produce citrinin such as *Penicillium viridicatum* (10² to 10³ CFU/100 cm²), *Penicillium citrinum* (10⁵ to 10⁶ CFU/100 cm²), *Penicillium lanosum* (10⁴ to 10⁵ CFU/100 cm²), and *Penicillium steckii* (10⁵ to 10⁶ CFU/100 cm²) (Klintworth et al., 1999). Importantly, high levels (10⁶ to 10⁷ CFU/100 cm²) of *Aspergillus flavus*, the producer of the carcinogenic AF, were reported (Klintworth et al., 1999).

Twelve species of the genera *Penicillium*, *Aspergillus*, *Cladosporium*, and *Aureobasidium* were isolated and identified from surfaces of Mir after 13 years of operation (Alekhova et al., 2005). More specifically, *P. chrysogenum* was the predominant species in all samples (71% of the samples, 43% of all fungal isolates). Furthermore, three species belonged to the genus *Aspergillus*, which are known producers of various toxic and carcinogenic mycotoxins and exhibit maximum adaptability to severe conditions. Additionally, *Cladosporium sphaerospermum* was also detected (Alekhova et al., 2005). The identified *Aspergillus, Penicillium, Cladosporium*, and *Aureobasidium* species in the study described above are correlated with various types of health effects related with mycoses (i.e., fungal infection of humans and animals) such as fever, nausea, skin irritation, allergic symptoms, aspergillosis, pneumonitis, nephrotoxicity, hepatotoxicity, and even carcinogenicity. Consequently, it is clear they present an important health risk. Another study isolated six strains from Mir and identified them as *A. versicolor, P. chrysogenum*, and other *Penicillium* species that are common saprophytic fungi in the environment and potentially pathogenic (Makimura et al., 2001). As mentioned above, some of these fungi are potential mycotoxin producers.

It has been stated that the microflora on board Mir consist of more than 100 species of microscopic fungi (Kozlovskii et al., 2002). The concentration of airborne fungi in Mir fluctuated between 2.0 × 10⁴ and 5.0 × 10⁴ CFU/m³, whereas contamination levels of surfaces and equipment were between 1.0 × 10² and 1.0 × 10⁷ CFU/100 cm² (Novikova et al., 2006). Respectively, *Penicillium spp.*, *Aspergillus spp.*, and *Cladosporium spp.* were detected in 76.8%, 39.4%, and 27.2% of the surface samples and were found in 75.8%, 76.6%, and 24.2% of the air samples, which makes *Penicillium* and *Aspergillus* dominant genera (Novikova, 2004). It appeared that resident strains of *P. chrysogenum* and *P. expansum*, which were isolated from materials on board Mir, synthesize a number of low-molecular-weight nitrogen-containing secondary metabolites of alkaloid origin such as roquefortine, meleagrin, rugulosuvin, and *N*-acetyltryptamine (Kozlovskii et al., 2002). Furthermore, a *P. expansum* strain isolated in Mir became dominant at the end of a long-term spaceflight and formed biologically active secondary metabolites identified as xanthocyllin X and questiomycin A, which are broad spectrum antibiotics (Kozlovskii et al., 2004). In another study, *P. expansum* Link and *P. chrysogenum* Thom strains were isolated at the Mir space station (Kozlovskii et al., 2009). Interestingly, the identification of these strains was based on the presence of the metabolites patulin, citrinin, and roquefortine C for *P. expansum* Link, and penicillins, roquefortine C, meleagrin, xanthocillins, and PR-toxin for *P. chrysogenum* (Kozlovskiy et al., 2009). Additionally, examination of free condensate samples during Mir 6 and 7 flights revealed *Acremonium sp.*, *Candida sp.*, *Cladosporium sp.*, *Fusarium sp.*, *Penicillium sp.*, and *Rhodotorula sp.* as the most commonly detected fungi (Ott et al., 2004).

Similar to the Russian space stations, examination of contamination inside the ISS revealed the presence of various fungal species. A study showed that all fungi identified in the ISS belonged to the same group of those sampled in Mir and were identified as *Penicillium* (3 species), *Aspergillus* (3 species), *Cladosporium* (1 species), and *Scopulariopsis* (1 species) (Alekhova et al., 2005). In this case, *Penicillium* was also the dominant genus (Alekhova et al., 2005). Species of the genera *Aspergillus* (particularly *A. flavus*), *Cladosporium*, and *Scopulariopsis* are opportunistic human pathogens. Additionally, fungi were obtained from...
A third study detected endotoxins including β-1,3-glucan (fungi-specific) as a marker of Gram-negative bacteria and fungi in 24 out of 42 surface areas tested on the ISS (Maule et al., 2009). Low levels (0.51–1.0 EU/100 cm²) were found on 13 surface areas, moderate levels (1.01–10.0 EU/100 cm²) at 10 areas, and high levels (10.1–24.0 EU/100 cm²) at one area (Maule et al., 2009). In general, moderate to high levels were found at sites mostly associated with hygiene, dining, exercise, or sleeping facilities (Maule et al., 2009). Satoh et al. (2011) reported that culture-dependent swab and microbe detection sheet investigation of orbital samples from the KIBO (Japanese experimental module on the ISS) were both negative. Similarly, microbe detection sheets (MDS) examined by emission-scanning electron microscopy revealed no microbial structures (Satoh et al., 2011). However, real-time PCR detected fungal DNA of Alternaria and Malassezia species as dominant before launch and in space, respectively (Satoh et al., 2011). As mentioned above, within the group of Alternaria species, some are able to produce mycotoxins. After Malassezia, Cladosporium was the next most dominant genus at the KIBO (Satoh et al., 2011). These Cladosporium species were also found, next to Neotyphodium species, in wheat plants that were grown on board the US space shuttle Discovery during an 8-day mission (Bishop et al., 1997). Approximately 50% of the 72 seedlings recovered after the mission showed signs of fungal contamination (Bishop et al., 1997). Finally, a more recent study isolated 37 fungal strains from six equipment locations on board the KIBO and from a space shuttle (Satoh et al., 2016). Similar to previously described studies, Penicillium, Aspergillus, and Cladosporium were the dominant species (Satoh et al., 2016).

In summary, the above-described results of fungal species that have been found in various space stations and spacecraft clearly indicate the highly important need to investigate mycotoxins in the space environment. Fungi that potentially produce AF, sterigmatocystin, patulin, OTA, and alternaria toxins were found on Russian space stations, the ISS, and shuttles. An overview of the detected mycotoxigenic fungi and their corresponding mycotoxins can be found in Table 1. This overview reveals that potential AF-producing species such as A. flavus are often found in space station samples, which is alarming since AFs are the most dangerous mycotoxins due to their proven carcinogenicity. Importantly, the presence of fungi does not necessarily mean mycotoxins are being produced. However, this possibility should be taken into account since there is currently no scientific information or data available on the impact of space conditions on the production of mycotoxins by fungi. Moreover, it is important to keep in mind that mycotoxins can still be present even when, over time, fungi are no longer detected. Additionally, the presence of low mycotoxin concentrations is not necessarily correlated to negative health effects. Regarding this point, TDI/TWI levels were set for some mycotoxins by the European Food Safety Authority (EFSA) and/or the European Commission, such as a TWI of 120 ng/kg body weight (bw)/week for OTA and a provisional TDI of 0.4 μg/kg bw/day for patulin (European Food Safety Authority, 2006b; Zhang et al., 2011). Since AFs are group I carcinogens according to the International Agency for Research on Cancer (IARC), a zero tolerance is prescribed, which is difficult to obtain since these toxins are
Table 1. Overview of Detected Mycotoxigenic Fungi on Board Russian Space Stations and the International Space Station (ISS), together with the Corresponding Mycotoxins That Can Be Produced by These Fungi

<table>
<thead>
<tr>
<th>Mycotoxigenic fungal species detected on board spacecraft</th>
<th>Mycotoxins</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. flavus, A. tamarii</td>
<td>Aflatoxins*</td>
</tr>
<tr>
<td>Alternaria alternata</td>
<td>Alternariol, alternariol monomethyl ether, alternate, tenuazonic acid</td>
</tr>
<tr>
<td>P. corylophilum, P. steckii, P. citrinum, P. lanosum, P. viridicatum, P. expansum</td>
<td>Citrinin</td>
</tr>
<tr>
<td>Fusarium spp.</td>
<td>Fumonisins and trichothecces</td>
</tr>
<tr>
<td>P. brevicompactum</td>
<td>Mycophenolic acid</td>
</tr>
<tr>
<td>Aspergillus ochraceus, A. niger, Penicillium viridicatum</td>
<td>Ochratoxin A</td>
</tr>
<tr>
<td>A. clavus, P. expansum</td>
<td>Patulin</td>
</tr>
<tr>
<td>P. purpurogenum</td>
<td>Rubratoxin B</td>
</tr>
<tr>
<td>A. versicolor</td>
<td>Sterigmatocystin</td>
</tr>
</tbody>
</table>

A. = Aspergillus; P. = Penicillium.
*Carcinogenic mycotoxins.

of natural origin. Therefore, EFSA set several maximum levels of, for example, 2.0 µg/kg for AF B1 and 4 µg/kg for the sum of AF B1, B2, G1, and G2 in ground nuts, dried fruit, and cereal products (European Food Safety Authority, 2006a). Consequently, monitoring mycotoxin concentrations on board a space station or vehicle is of high importance to ensure that TDI/TWI and maximum levels are not being exceeded during space missions.

4. Sources and Prevention of Fungal Contamination in Space Stations

Most likely, contamination of space stations originates from materials associated with preflight manufacturing and assembly, the delivery of supplies to space stations, the supplies themselves, and secondary contamination from the crew or other biological materials on board (Novikova et al., 2006). In fact, the crew itself is probably the most important contamination source by way of air, sedimentation, and direct transfer to surfaces (Novikova et al., 2006). Pathogenic microflora may propagate on these surfaces from which crew members may be infected directly or indirectly through air or water (Klintworth et al., 1999). With respect to mycotoxins, it can be assumed that the major mycotoxin exposure route in space will most likely be through skin contact with infected substrates and inhalation of toxins (Bennett and Klich, 2003). Fungal species such as Penicillium and Aspergillus can significantly produce fungal volatiles under indoor conditions, which has been related to the sick-building syndrome on Earth (Polizzi et al., 2012). This syndrome refers to symptoms experienced when a building is characterized by poor indoor air quality (Polizzi et al., 2012). Since space stations are relatively small, closed systems in which astronauts are continuously enclosed, a comparable syndrome might occur.

To date, no known fungi-related health effects have been publically reported as a result of living on board the ISS (Vesper et al., 2008). Nevertheless, it is clear that there is still a need for continual assessment of fungal development, mycotoxin production, and efficient contaminant prevention and contamination remediation strategies (Alekhova et al., 2005).

Several preventive measures are currently taken to avoid contamination. NASA aims to prevent microbial growth by several measures such as reducing humidity, eliminating free water, implementing routine housekeeping, and food monitoring (Yamaguchi et al., 2014). More specifically, the air inside the ISS is continuously filtered to meet the established limits, surface and water samples are collected regularly, and the sterility of commercially sterile food is checked preflight (Van Houdt et al., 2012b). Additionally, molecular-based methods are, for example, being used during assembly to ensure that important spacecraft surfaces are clean and sterile (La Duc et al., 2004, 2012). In general, great effort is made to prevent fungal contamination on board the ISS such as avoiding condensation on surfaces and keeping relative air humidity levels below 70% (Vesper et al., 2008). Additionally, HEPA filters are applied in the air system, and all external modules delivered to the ISS are assembled in clean rooms and disinfected with hydrogen peroxide (3–6%) (Vesper et al., 2008). The addition of iodine (US system) or ionic silver (Russian system) to the water is a final example of a preventive measure (Artemyeva, 2016). Importantly, it should be noted that bacterial resistance to ionic silver has already been described (Percival et al., 2005; Sutterlin et al., 2017). Therefore, the possibility exists that this process can also occur in fungi when ionic silver is overused (Percival et al., 2005).

Despite the above-described preventive measures, additional specific methods to inhibit and prevent fungal growth and corresponding mycotoxin production should be investigated and developed. Research such as that performed by Devarayan et al. (2015) is important in that it assesses the inhibition of Aspergillus niger growth under microgravity by 2-amino phosphonate chitosan. This compound is able to inhibit fungal growth under microgravity and reduce production of secondary metabolites (Devarayan et al., 2015). As a result, a biobased chitosan derivative could be used as a disinfectant and preventive agent in space stations for the suppression of mycotoxins in microgravity conditions (Devarayan et al., 2015).

5. Monitoring Fungi and Mycotoxins in Space: Application of Various Detection Methods

It is clear that microbial monitoring is highly important to ensure the safety of a space crew, especially in long-term missions, and maintain the efficiency of life-support systems in space (Yamaguchi et al., 2014). Fortunately, monitoring microorganisms in air, water, and on surfaces before, during, and after spaceflights is a routine endeavor (Maule et al., 2009; Yamaguchi et al., 2014). For example, microbiological examinations were an important aspect of the Health Stabilization Program in conjunction with the US space shuttle (Klintworth et al., 1999). Various techniques exist to monitor the microbiological burden on space stations (Van Houdt et al., 2012a).
Historically, the detection of microorganisms in spacecraft was, and is today, accomplished with the implementation of culture-dependent methods. Typically, samples are collected by astronauts and, in some cases, incubated on board, and colony counts are reported. Subsequently, the samples and/or cultures are sent to Earth for identification of the microbes (Vesper et al., 2008). Surface sampling involves swabbing and air acquisition with an air sampler (Makimura et al., 2001; Novikova et al., 2006). After the return of all samples to Earth, aliquots are inoculated on Petri dishes that contain various nutrient media, and fungal concentrations are determined and strains identified microscopically (Novikova et al., 2006). *Penicillium* fungi isolated from the Russian Mir space station were grown on a mineral Abe medium that contained succinic acid and mannitol as carbon sources with subsequent isolation, purification, and identification of metabolites (Kozlovsky et al., 2009). The NASA standard Assay and Plate Count Assay have become culture-dependent methods that have been used for the ISS (La Duc et al., 2004). The former assay aims for enumeration of spores and heterotrophic microbial populations in a swab sample that is placed into a sterile phosphate-buffered rinse solution that is split into two parts, one of which is subjected to heat shock (La Duc et al., 2004). Aliquots are placed in Petri dishes, and total aerobic counts are determined by the pour plate technique with the use of tryptic soy agar (TSA) as a fungal growth medium (La Duc et al., 2004). The latter assay is used for the cultivation of mesophilic and heterotrophic microbial populations in which samples are plated onto pour R2A agar plates (La Duc et al., 2004). Also, a kit called Biopros has been used for taking samples from Mir and from surfaces of the Russian section of the ISS (Alekhova et al., 2005). In this study, samples were taken in space, followed by microbiological isolation and analysis on Earth through plating on agar nutrient media (Alekhova et al., 2005). Other methods were used for swabs collected from the Japanese KIBO section of the ISS, which were placed in activated charcoal agar, and MDS were cultured in the ISS and fixed for electron microscopy (Satoh et al., 2011). Furthermore, microbial monitoring of the ISS surfaces is often performed with contact slides to detect growing characteristics of microorganisms on a certain type of agar-based media (Maule et al., 2009). These slides are held to the surface, and after some days colony growth can be detected (Maule et al., 2009).

Conventional culture-dependent methods are, however, characterized by a number of disadvantages (Novikova et al., 2006). An important main disadvantage is related to the actual culturing of microbial cells and thus potential pathogens/toxins in space, which includes a significant health risk. Furthermore, only about 1% of the microorganisms reported are cultivable, and cultivation-dependent methods rely on prior knowledge and expertise of the microbes (La Duc et al., 2004). Moreover, returning samples to Earth for analysis generates a time gap between sampling and availability of results (Novikova et al., 2006).

Developing on-line monitoring and on-site analysis systems such as the Microbial Detection in Air System for Space (MiDASS) is highly important to be able to alert cosmonauts at an early stage about an increase in biological contamination so that appropriate countermeasures can be taken in time (Novikova et al., 2006). Second, an enhanced characterization of microbial contamination without amplifying the risk by using culture-independent molecular analysis is preferable. In this regard, several biomarkers such as adenosine triphosphate (ATP), lipopolysaccharide, and DNA have been targeted to quantify total microbial contamination or specific types thereof (La Duc et al., 2004).

By using ATP-based microbial detection, intracellular ATP (viable microbes) as well as total ATP (extracellular and intracellular) can be measured (La Duc et al., 2004). To determine total ATP, a sample is combined with a cell lysing detergent and a luciferin-luciferase reagent to measure the amount of bioluminescence with a luminometer (La Duc et al., 2004). To determine intracellular ATP, an extracellular ATP-eliminating reagent is added prior to the assay described above (La Duc et al., 2004). Another technique is based on the *Limulus* amoebocyte (LAL) assay, which is an enzyme-based colorimetrical quantitative test to detect endotoxins as markers of Gram-negative bacteria and fungi (Maule et al., 2009). The system based on this LAL assay, called Lab-On-a-Chip Application Development Portable Test System (LOCAD-PTS) was developed to overcome limitations of contact slides (Maule et al., 2009). LOCAD-PTS is a handheld spectrophotometer that uses the LAL assay to detect endotoxins on surfaces and provides results within 15 min, which are readable on a LED display (Maule et al., 2009).

LOCAD-PTS cartridges have been launched to the ISS for specific detection of β-1,3-glucan (fungi-specific) (Maule et al., 2009). Importantly, this LOCAD-PTS system enables detection of endotoxins, which is not possible through fungi contact slides (Maule et al., 2009). Next, for DNA-based fungal detection, DNA is extracted directly from samples, and eukaryotic 18S rRNA genes are amplified with NS1F and NS8R primers (La Duc et al., 2004). By using these types of techniques, both cultivable and noncultivable microbes are confirmed, and quantitative PCR approaches can be used to detect and quantify microbial species (La Duc et al., 2004). However, it should be noted that only one cultivable fungal species (i.e., *Aureobasidium*) was found in this study (La Duc et al., 2004).

A first example of a DNA-based method for mold analysis is called mold-specific quantitative PCR, which has been developed to identify and quantify species found in dust collected from HEPA filters inside the US Laboratory Module of the ISS (Vesper et al., 2008). Second, isolated strains from the KIBO were also identified by comparison with DNA sequences (Satoh et al., 2011, 2016). Finally, molecular identification using 18S- and ITS1-rDNA sequences of fungi isolated on board Mir was performed in conjunction with the previously described morphological techniques (Makimura et al., 2001). Another culture-independent technique is found in the “electronic nose,” which is based on metal oxide sensors that provide rapid detection of fungi on ISS materials (Warrelmann et al., 2005). This detection method characterizes microorganisms by their odor profile with short measuring times that result in a differentiation of fungal species and identification of the growth stage (Warrelmann et al., 2005). Microscopy is the last culture-independent method described, which is used for qualitative assessment, although it has some limitations such as autofluorescence (La Duc et al., 2004). Therefore, it is impractical to use in this setting due to the generally low concentrations of microbes that occur on spacecraft (La Duc et al., 2004).
In summary, both culture-dependent and culture-independent techniques have been described and compared for the examination of the abundance of microorganisms from spacecraft and associated environments. Knowledge of cultivable as well as total microbial burden is valuable (La Duc et al., 2004). However, populations are generally found to be more diverse when examined by molecular-based methods than by culture-dependent methods, given that these assays show no preference to cultivable species and detect the presence of all contaminating microbes (dead and alive) (La Duc et al., 2004). Molecular assays are particularly applicable for monitoring samples from environments with low microbial levels, as can occur in spacecraft (La Duc et al., 2004). The power of rapid techniques is the possibility of combined use to gain additional and general insight into the microbial population (La Duc et al., 2004), together with the ability to take countermeasures such as decontamination strategies in time. Therefore, the development of new rapid, real-time, and onboard techniques such as portable microfluidic systems is of high importance (Yamaguchi et al., 2014).

Identification of potential mycotoxin-producing fungi by means of several monitoring methods does not result in any conclusive information concerning the presence of mycotoxins. Therefore, monitoring of mycotoxins requires specific methods in addition to those already applied to identify fungi. As mentioned before, mycotoxin levels in space have not yet been monitored according to our knowledge. Consequently, no specific detection methods have been described to monitor these molecules in a space environment. On Earth, several studies involved detection of mycotoxins such as OTA, zearalenone, deoxynivalenol, sterigmatocystin, fumonisins, and AF derivatives in air through an aerosol/air sampler, followed by an extraction procedure and liquid chromatography with fluorescence or (tandem) mass spectrometry detection (Bloom et al., 2007; Wang et al., 2008; Polizzi et al., 2009; Torelli et al., 2010; Jargot and Melin, 2013; Sanders et al., 2014). Similarly, air and dust samples can be taken on space stations, returned to Earth, and subsequently analyzed to determine the mycotoxin content. Another possibility is the use of rapid tests for the detection of multiple mycotoxins such as immunoassays (Beloglazova et al., 2017; Foubert et al., 2017). However, the efficient use of these tests on board spacecraft to analyze air and surfaces has not been proven to date. Most likely, these methods will require several modifications prior to their use in space.

6. Strategies to Control Fungal and Mycotoxin Exposure in Space: Limits and Decontamination

The above-illustrated presence of fungal growth and the existing risk of mycotoxin exposure clearly indicate the importance of controlling fungal occurrence in space by setting limits and developing decontamination strategies. Both the US and Russian space programs have indicated that fungal species like Aspergillus fumigatus and Cryptococcus neoformans have to be avoided on material surfaces or in air (Klintworth et al., 1999). In this respect, maximum allowable concentrations for contamination, as well as requirements concerning sampling locations and frequency of monitoring, are articulated in the International Space Station Medical Operations Requirements Document (ISS MORD SSP 50260) (International Space Station Program, 2003; Van Houdt et al., 2012a). For surfaces, preflight limits regarding fungal contamination of internal surfaces have been defined as <10 fungal CFU/100 cm², whereas inflight requirements are defined as <100 fungal CFU/100 cm² (International Space Station Program, 2003; Yamaguchi et al., 2014). For air, limits were set at 50 fungal CFU/m³ and 100 CFU/m³ for preflight and inflight, respectively (International Space Station Program, 2003; Yamaguchi et al., 2014).

With respect to mycotoxin health-based guidance values, no TDI/TWI levels have been set, to our knowledge, for mycotoxins present in specific foodstuffs, on surfaces, and in air for the ISS. Moreover, it can be assumed that all food supplies provided from Europe (ESA) that are destined for delivery to the ISS will have to comply to European standards and, thus, to the TDI/TWI levels for mycotoxins set by EFSA as previously described in this review (e.g., TWI of 120 ng/kg bw/week for OTA [European Food Safety Authority, 2006b; Zhang et al., 2011]). Importantly, differences can be found between TDI/TWI values set by different countries that contribute to the ISS such as Europe, Japan, the United States, and Canada. For example, Europe and Japan apply similar levels for OTA, whereas a lower TDI value of only 4 ng/kg bw/day is accepted by Canada (Food Safety Commission of Japan, 2013; Picó, 2008). Therefore, it will be necessary to provide health-based guidance values that are internationally accepted. Moreover, there should be a focus on foodstuffs that are sent to space such as specific dried or canned foods. Even though food is mostly sterilized before spaceflight, it remains possible that mycotoxins can survive this treatment and cause health problems.

In addition to setting the above-stated limits, several countermeasures have been developed to be implemented on board space stations to restore the quality of surfaces, air, and food in case of contamination events (Van Houdt et al., 2012b). However, restoring the quality of water seems not so straightforward. To our knowledge, if water contamination occurs, the water tank will be treated as waste, so currently no successful countermeasures exist for water contamination problems. For decontamination of surfaces or air, hydrogen peroxide liquid or vapor is used on Earth. However, in space this is not possible, since liquids and vapors can cause problems, as droplets may fly around. Therefore, if in space the contamination levels on surfaces are above the acceptability limits, a cleanup by the crew can be performed by using quaternary ammonium disinfectant wipes (Vesper et al., 2008). With regard to air, the Russian POTOK decontamination system for destruction of airborne microorganisms can be applied and has already been installed in the American segment of the ISS (Kapustina and Volodina, 2004; Whitworth, 2017). However, it remains uncertain whether these wiping and POTOK systems are effective for mycotoxin degradation, as the POTOK system only applies an electric field to create pores in cell membranes (Whitworth, 2017).

7. Recommendations for Future Research and Development

The above-described observations concerning the presence of fungal species in space clearly show that strict contamination prevention procedures and continuous preflight and inflight monitoring are highly important to ensure
the astronauts’ health on board space stations. Although no real negative health effects have been publically reported to date as a result of fungi in space, the potential presence of mycotoxins can result in a number of critical health risks for astronauts, especially through chronic exposure over long-duration missions.

Although it has been said that contamination levels are generally lower than established limits, some reports indicate that further improvement of prevention and detection methods is advisable (Van Houdt et al., 2012b). Detection methods for monitoring mycotoxins on surfaces and in air in space should be developed. As a first step, this could be accomplished by the implementation of methods that are applied on Earth to measure mycotoxins in dust and air, which was described above with regard to the monitoring of fungi and mycotoxins in space. Secondly, new rapid tests that enable onboard detection of mycotoxins would be of significant interest and should be developed. Moreover, it is highly recommended to continue investigation toward mycotoxin production by fungi present in space, under the specific environmental conditions that prevail on space stations (e.g., specific use of materials and chemicals, reduced gravity, increased cosmic radiation doses). As is the case for astronauts, microorganisms such as fungi are exposed as well to various types and doses of radiation in space. The impact of this radiation on these fungi and their potential mycotoxin production is thus an important aspect to be considered. Generally, NASA has indicated that the average exposure of astronauts on the ISS or a shuttle is estimated to be between 80 and 160 mSv for 6 months (Lyndon B. Johnson Space Center, 2002). To illustrate, on Earth each person is exposed to a background radiation of approximately 2 mSv per year (Lyndon B. Johnson Space Center, 2002); thus, on the ISS, doses are about 80–160 times higher than those on Earth. For example, an average dose of 180 μGy/day was measured in the low Earth orbit of the ISS, which is 80-fold higher compared to the background radiation level on Earth (Goossens et al., 2006; Vanhavere et al., 2008; Mastroloeo et al., 2009). Several studies have addressed the impact of ionizing radiation on fungi and mycotoxin production. In fact, on Earth, ionizing radiation is the most-used decontamination and detoxification strategy for fungi and corresponding mycotoxin production in food (El-Samahy et al., 2000). In this regard, mostly high acute doses of gamma radiation are administered to food. Irradiation of food with high doses will result in killing microorganisms, including fungi. However, results on the actual influence of such irradiation on fungi and mycotoxin production are conflicting. On the one hand, several studies state that gamma irradiation of food can be used to decrease fungal growth and mycotoxin levels based on experiments performed with several Aspergillus, Penicillium, Fusarium, and Cladosporium species, including, among others, A. flavus, A. niger, A. ochraceus, A. parasiticus, P. expansum, and P. citrinum (El-Samahy et al., 2000; Aziz and Moussa, 2002, 2004; Aziz et al., 2006; Yun et al., 2008; Jalili et al., 2012; Ahsan et al., 2013; Di Stefano et al., 2014; Markov et al., 2015; Kanapitsas et al., 2016). On the other hand, some studies argue that mycotoxin production by, among others, A. flavus, A. ochraceus, and Fusarium culmorum is enhanced by gamma irradiation, and sub-inhibitory doses could cause stimulation of fungal sporulation and growth (O’Neill et al., 1996; Ribeiro et al., 2009, 2011). However, the studies mentioned above investigated the effect of mostly acute high-dose gamma radiation (e.g., 0.5–30 kGy) during a rather short time frame on fungi and mycotoxin production, while the radiation doses in space are much lower compared to the ones applied in these studies. To analyze the effect of radiation that occurs in space stations, more in-depth research will be needed on a more chronic exposure to lower doses. Moreover, in most investigations along these lines, only one type of radiation is considered, such as gamma radiation, although in spacecraft astronauts and fungi are exposed to a complex field of cosmic radiation. Therefore, irradiation experiments on Earth will need to be designed such that they mimic as well as possible real space conditions, though it would be advantageous as well for such investigations to take place in space to elucidate the influence of radiation exposure on fungi and mycotoxin production. In this respect, the effect of varying types of radiation on the same fungal species, as was recently performed in multiple studies by Pacelli et al. (2017), is an important contribution that will help elucidate irradiated fungi behavior (Pacelli et al., 2017, 2018). Obviously, radiation is not the only parameter in space that can alter mycotoxin production. Several other environmental factors have an important influence on toxin production such as oxygen concentration, the type of substrates (surfaces) used in spacecraft, competing microflora, temperature gradients in space cabins, and different gravitational conditions (Verma, 2007). Consequently, when mimicking space conditions to investigate the influence on fungi and mycotoxin production, these parameters should be examined as well. As an example, candidate materials for use in space missions need to be evaluated for their susceptibility to fungal biofilm formation and biodegradation (Gu, 2007). With respect to the competing microflora, it is important to keep in mind that microbial contamination exhibits a change of the dominating species by quantity and prevalence during space missions (Novikova, 2004). Moreover, since the spacecraft environment is also characterized by microgravity and higher radiation compared to Earth conditions (Lyndon B. Johnson Space Center, 2002; Checinska et al., 2015), researchers could model these conditions as well by performing experiments on Earth in simulated microgravity.

Finally, more efforts are needed to develop additional effective fungal and mycotoxin decontamination procedures as countermeasures against potentially alarming contamination levels. Often, mycotoxins are stable compounds that cannot easily be degraded. Hence, current countermeasures in use today may not be sufficient when applied under space conditions. Some research has been conducted with regard to the effect of UV radiation of which the higher UV part in the spectrum is also defined as a type of ionizing radiation that could be administered to surfaces via lamps or torches to decontaminate surfaces. However, as is the case for gamma radiation, no consensus has been reached with respect to the effect of radiation on fungi and mycotoxin production (Aziz and Smyk, 2002; Paterson and Lima, 2011; Garcia-Cela et al., 2015). Importantly, no clear correlation has been found between the observed effects and the dose of radiation used. Furthermore, it appears that the effects of radiation are highly dependent on the type of samples. More research is needed to examine the actual
fungal decontamination and mycotoxin detoxification potential and applicability of UV radiation in space.

8. Conclusions

The presence of various fungal species on board space stations has clearly been confirmed, although fungal contamination does not appear to be alarming. However, contamination should not be underestimated, since it has the potential to cause health risks on long-term missions (Klintworth et al., 1999). Moreover, the risk of health-related problems could increase as flight durations increase due to an increasing load of microorganisms (Klintworth et al., 1999; Gu, 2007). Importantly, several detected fungi in spacecraft are potentially mycotoxigenic and thus capable of producing mycotoxins that would be a threat to human health. Despite these serious health risks, no studies, to our knowledge, have clearly investigated mycotoxin production and the presence of mycotoxins on space stations. The behavior of fungi in space conditions and their potentially corresponding mycotoxin production are, at present, unknown.

This review clearly emphasizes that it is important to prevent and reduce the presence of fungi on space stations as much as possible so as to ensure the health of astronauts and the integrity of space materials. Although a number of preventative, monitoring, and decontamination techniques are currently available that aid in the reduction and investigation of fungal contamination, the development of new detection and decontamination methods will be required to reduce the potential for astronaut exposure to fungi and mycotoxins in space.

In conclusion, the threat of fungi and mycotoxins in space will require more in-depth research on Earth and in the space environment.

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Author Disclosure Statement

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References


European Food Safety Authority. (2006b) Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to ochratoxin A in food. *EFSA J* 365:1–56.


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<table>
<thead>
<tr>
<th>Abbreviations Used</th>
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<tbody>
<tr>
<td>AF = Aflatoxin</td>
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<tr>
<td>ATP = adenosine triphosphate</td>
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<tr>
<td>bw = body weight</td>
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<tr>
<td>CFU = colony-forming units</td>
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<td>EFSA = European Food Safety Authority</td>
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<tr>
<td>EU = endotoxin units</td>
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<tr>
<td>ISS = International Space Station</td>
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<tr>
<td>LAL = Limulus amoebocyte</td>
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<tr>
<td>LOCAD-PTS = Lab-On-a-Chip Application Development Portable Test System</td>
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<tr>
<td>MDS = microbe detection sheets</td>
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<tr>
<td>OTA = ochratoxin A</td>
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<tr>
<td>TDI = tolerable daily intake</td>
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<tr>
<td>TWI = tolerable weekly intake</td>
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