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Prevalence of Fungi During Skylab Missions

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Samples for mycological analysis were collected from surfaces in the Skylab spacecraft before launch and during flight for each manned mission. Fungal contamination levels were low during the first two flights; however, the species recovered were different for each mission. On the third mission, widespread contamination of the Skylab spacecraft with *Aspergillus* and *Penicillium* spp. was detected. This contamination was traced to several contaminated space suit undergarments.

Before the first Skylab mission, it was anticipated that during long-term space flights, moisture accumulation might occur in the spacecraft and result in excessive growth of molds (1, 7). Local proliferation of molds was observed during a simulated Skylab mission, and it was felt that such excessive mold growth could have an impact on the health of the crew or result in degradation of materials in the spacecraft (7).

During the Apollo space flights, microbial samples collected from the command module before and after missions did not suggest increases in fungi (4, 5). However, these missions of approximately 14-day duration were much shorter than those of Skylab. Furthermore, the Skylab spacecraft presented a much more complex environment than the smaller Apollo vehicle. Each Skylab flight was provided with materials for the collection and storage of microbial samples. This paper describes an increased occurrence of molds detected in these samples and provides a mycological profile of the Skylab spacecraft.

MATERIALS AND METHODS

Mission schedules and environment. The Skylab program began with the launch of the unmanned Skylab spacecraft (designated SL-1) which remained in orbit for subsequent visitation by three different astronaut crews (designated SL-2, SL-3, and SL-4), each composed of three astronauts. The duration of each flight was 28, 59, and 84 days, respectively.

The Skylab atmosphere was maintained at approximately 70% O_2 and 30% N_2 at a nominal pressure of 259 mm of Hg except during unmanned phases of the program when the ambient pressure was allowed to drop to 1/10 of this value. Temperatures during the unmanned SL-1 flight rose briefly to a high of 54.4°C due to the loss of a micrometeoroid shield during launch. After correction of this problem, however, temperatures remained within an approximate 19 to 27°C range during the manned visitations. The spacecraft environmental control system was designed to maintain a relative humidity of 50% or lower, and the highest humidity recorded on any mission was 53%.

While occupying the space vehicle, the crew performed regular clean-up and maintenance protocols, including trash and waste food disposal. Routine personal hygiene schedules included showers and were followed by each crew member.

Samples collected. A single set of preflight environmental samples was collected from the Skylab 35 days before launch of the unmanned vehicle. Subsequent samples were collected twice during each manned flight. Collection dates were scheduled approximately 2 and 14 days before splash down (see Tables 1 and 2) in an effort to standardize sample storage during each mission. At each of the sampling periods, 15 specimens were collected from inhabited areas of the spacecraft. Areas examined included surfaces on in-flight experiment gear, dining areas, trash disposal equipment, waste management areas, bulkheads, switch panels, and hatch covers.

Each sample was collected with a sterile calcium alginate swab (Whirlpool Corp., St. Joseph, Mich.) moistened in sterile distilled water. Crewmen were instructed to scrub a 25.8-cm^2 area at each identified site. The head of the swab was then placed in a 5-ml vial of Stuart medium base (Baltimore Biological Laboratory, Cockeysville, Md.) containing 0.6% agar and broken well below the area held by the crewman. Samples were capped, stored at 4°C during each flight, and returned to earth in a chilled container.

Specimen analysis. All samples were processed on board the recovery ship within 3 h after splash down. Initial sample processing was accomplished in a laminar flow hood.

The contents of each vial were transferred to a sterile tube containing glass beads. The vial was rinsed twice with 2.5 ml of veal infusion broth (Baltimore Biological Laboratory), and the washings were combined with the original contents of the vial. The sample was then mixed thoroughly with a Vortex mixer, and 5 ml was removed for mycological analysis. Further sample processing and identification of fungi were

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performed by the methods described by Taylor et al. (6).

RESULTS

Twenty-one species of fungi were recovered from Skylab missions (Table 1). The number of species found increased with each succeeding flight; *Aspergillus* and *Penicillium* spp. were the most common fungi isolated. However, the pattern of the species recovered was unique for each flight. *Periconia lateralis* was isolated before launch but was not recovered from later samples. *Aureobasidium pullulans* was the predominate species recovered from SL-2, whereas *Penicillium* spp. and *Candida parapsilosis* predominated on SL-3. A wide variety of *Penicillium* and *Aspergillus* spp. was found in samples from SL-4. Only three filamentous fungi, *Aspergillus flavus* var. *columnaris, Penicillium notatum, Cladosporium sphaerospermum*, and two species of yeast, *C. parapsilosis* and *Cryptococcus laurentii* var. *laurentii*, were recovered from more than one flight. No yeasts were recovered from SL-1 or SL-2 samples.

TABLE 1. *Fungal isolates from 15 sites in the Skylab spacecraft*

Organism	No. of sites positive						
	SL-1	SL-2		SL-3		SL-4	
	L − 35[a]	L + 16	L + 27	L + 45	L + 57	L + 69	L + 81[b]
Aspergillus chevalieri						1	
A. flavus var. *columnaris*			1				1
A. phoenicis						2	
A. pulvinus						1	2
A. sydowi						6	2
A. terreus						1	
A. unguis						2	2
A. versicolor						3	4
Aureobasidium pullulans		4	1				
Cladosporium sphaerospermum				1		2	
Microascus intermedius							1
Penicillium corylophilum						1	1
P. decumbens					1		
P. funiculosum				1			
P. granulatum					2		
P. notatum					2	5	4
P. variabile							1
Periconia lateralis	1						
Candida parapsilosis				2	3		3
Cryptococcus laurentii var. *laurentii*					2	1	1
Rhodotorula rubra						1	1
Total sites positive for:							
Molds	1	4	2	2	3	11	7
Yeasts	0	0	0	2	5	1	3

[a] Sample day (launch ± number of days).
[b] Only 14 samples were obtained.

The extent of fungal contamination of the Skylab was estimated from the number of times a species was isolated, the total number of samples positive for fungi (Table 1), and the average number of species found in each sample (Table 2). Quantitation of fungi in samples collected by swab and subjected to agitation was not attempted due to the colony-forming potential of both mycelial fragments and spores. Thus, these results are presented only in terms of positive samples and number of species per sample. Recovery of fungi was not expected to be adversely affected by the maximum 15-day storage periods encountered in Skylab.

A binomial test (8) of the distribution of samples positive and negative for fungi between the two sampling periods on each mission revealed that recovery of fungi from samples collected at the end of missions did not differ significantly ($P > 0.05$) from samples collected earlier in corresponding missions. Fungal isolation rates for different missions were compared using Cochran's Q test (8). This comparison revealed that, despite the absence of yeasts in SL-1 and SL-2 samples, the frequency of yeast isolation did not differ significantly between any of the flights. Although filamentous fungi were recovered with similar frequency from SL-1, SL-2, and SL-3 samples, the incidence of positive samples was significantly ($P < 0.05$) different on SL-

TABLE 2. *Mean number of fungal species at 15 sites in the Skylab spacecraft*

Type of organism	Avg no. of species recovered per sample site						
	SL-1	SL-2		SL-3		SL-4	
	L − 35[a]	L + 16	L + 27	L + 45	L + 57	L + 69	L + 81[b]
Mold	0.07	0.3	0.1	0.1	0.3	1.7	1.2
Yeast	NR[c]	NR	NR	0.1	0.3	0.1	0.3

[a] Sample day (launch ± number of days).
[b] Only 14 samples were obtained.
[c] NR, None recovered.

4. This difference is attributed to a sharp increase in the average number of molds found at each site on SL-4 (Table 2). The reason for this increase was apparently related to the unpacking of several "mildewed" space suit liquid-cooled undergarments (LCG) early in the SL-4 mission. In anticipation of potential effects on environmental samples which were obtained later in the mission, one of the garments was returned to earth and sampled at 12 sites with direct-contact plates. Because samples obtained in this manner are not subject to extensive mixing, actual colony counts are presented (Table 3) as an approximation of the abundance of each species on the LCG. The molds isolated from the garment were primarily *Aspergillus* and *Penicillium* spp. *Aspergillus sydowi*, *Aspergillus unguis*, and *Penicillium notatum* were the most common molds on the LCG and corresponded to the three most frequently isolated species from SL-4 samples (Table 1).

DISCUSSION

Fungi were commonly isolated from preflight samples of Apollo spacecraft (3–5) and have been reported from postflight samples of at least one Apollo command module (5). These early data led to speculation that excessive growth of molds, particulary in areas of high humidity, might occur in the larger and more complex Skylab spacecraft (1, 7). In a simulated Skylab mission, apparent mold growth was observed in the exhalation hose of a metabolic analyzer and in the condensation around a food chiller (7). The potential for allergic reactions or pulmonary infections due to inhaled spores and for degradation of materials in the spacecraft was recognized (7). These considerations resulted in a program to evaluate in-flight mycological contamination of the Skylab environment. Contrary to predictions, excessive mold growth was not noted in the data obtained from the first two missions. On the third mission (SL-4), however, increased mold contamination was indicated both by distribution of fungi in the spacecraft and by the diversity of species recovered. This increase appeared to be directly attributable to mold growth on four LCG.

TABLE 3. *Fungi isolated from 12 areas on an SL-4 LCG*

Organism	Colonies	
	No.	% of total
Alternaria alternata	2	0.06
Aspergillus ficuum	6	0.2
A. flavus var. *columnaris*	214	6.3
A. foetidus	4	0.1
A. oryzae	22	0.6
A. phoenicis	81	2.4
A. sydowi	712	20.8
A. terreus	239	7.0
A. unguis	614	17.9
A. ustus	191	5.6
A. versicolor	258	7.5
Chaetomium globosum	1	0.03
Microascus intermedius	2	0.06
Paecilomyces parvus	4	0.1
Penicillium corylophilum	272	8.0
P. ochraceum	10	0.3
P. notatum	632	18.5
P. vermiculatum	158	4.6

On mission day 6 of SL-4, the crew unpacked all four available LCG on board and reported them "damp and mildewed." One LCG had been stored in the spacecraft since the beginning of the SL-2 mission (182 days of storage), whereas the remaining three had been stored since the SL-3 mission (118 days). Because the LCG had been sealed in moisture-impervious containers before launch and were not opened before day 6 of the SL-4 flight, it is unlikely that contamination took place during the SL-2 or SL-3 missions. Apparently, the aqueous liquid coolant from the LCG created a highly humid atmosphere within the stowage containers, allowing spores initially present to germinate and grow during the lengthy storage period. LCG used on SL-2 and SL-3 were not reported mildewed, but fresh LCG were supplied for these missions and had not been subjected to extensive storage before use. Suitable fungicidal cleaning agents were not available to the astronauts, and spores were doubtless disseminated widely each time the contaminated garments were used. Analysis of an LCG returned for sampling revealed that it was heavily contaminated with *Aspergillus* and *Penicillium* spp., and the species recovered corresponded very closely to those recovered from the Skylab environmental samples (Tables 1 and 3). These findings, together with the history of the LCG, confirm that these garments were the source of the molds recovered from SL-4.

With the exception of the abundance of molds on SL-4, fungi were absent in most samples from other Skylab missions. Many of the species recovered from SL-2 and SL-3 were also isolated from Apollo crewmen (5, 6), and their occurrence in the Skylab probably represents microflora carried on board by the crews. SL-2 yielded the fewest mold species of the three manned missions, and the absence of yeast in samples from this mission is consistent with this pattern. The relatively low frequency of samples positive for fungi in the data from SL-1 and SL-2 may be attributable to the cleanliness maintained in the Skylab before launch, to the regular housekeeping protocols observed during each mission, to

the low humidity recorded on each flight, and perhaps—in part—to the high temperatures experienced during the unmanned SL-1 mission. Only three species of molds were found in samples from more than one mission. Thus, each manned mission was characterized by an essentially different fungal microflora.

The results described here represent the first documented instance of excessive and potentially detrimental microbial growth in the spacecraft environment during a flight. Although acute medical problems did not result and the mold-contaminated LCG remained usable throughout the mission, the excessive presence of molds was not without impact. One instance of probable skin infection was reported on SL-4 (2). This infection, which may have resulted from the wearing of contaminated LCG, responded to treatment with Tinactin (2). Furthermore, the high levels of fungi in the spacecraft were apparently responsible for the gross contamination observed in a microbial growth experiment which was executed after the first use of the LCG. Samples from these contaminated culture plates, obtained on recovery day, revealed predominantly the *Aspergillus* and *Penicillium* spp. recovered from SL-4 environmental samples and from the LCG.

Fortunately, the overall mission impact of the increased mold contamination of SL-4 was minimal and resulted in only minor discomfort and some data loss. However, severe allergic reactions, infections, or extensive material degradation could have seriously compromised the mission. Similar contamination problems should be avoided on future space flights.

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