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Fungal colonization of synthetic substrates for use in space craft

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SUMMARY

Materials being used or considered for use in space flights were examined for their susceptibility to fungal colonization. The materials included soft goods (clothing) and insulation and fabrication products such as Velcro® attachments and elastic cord binders. Materials were exposed for at least 28 days in a high-humidity chamber colonized with over 50 species of fungi, including those species recommended for determining recalcitrance of materials to fungal biodegradation. At least nine of 25 products demonstrated extensive microscopic colonization by fungi, mostly by *Acremonium obclavatum*. Challenge procedures that rely on observations with the unaided eye, or 40 × magnification of growth by a restricted number of fungal species with a cellulosic substrate as a positive control, are insufficient for determining the resistance of synthetic substrates to fungal colonization.

INTRODUCTION

Fungi are integral constituents of the human environment, and they affect human life in many ways [6]. Interiors of the proposed international space station or any other extraterrestrial human habitat cannot be maintained germ-free. Crew exchange, payload and on-board plant and animal experiments contribute to the environmental microbial load. Fungi have been frequently isolated from the space shuttle interiors (Pierson, unpublished data), and the Russian space station Mir has had serious problems with fungal colonization of the inner surfaces and equipment (A.N. Viktorov, personal communication).

Because moisture and nutrients are absorbed by surfaces, most interior surfaces in spacecraft will be subjected to colonization by microorganisms, including fungi. Amplification of fungi on complex substrates may result in deterioration of materials, instrument malfunction, production of volatile organic compounds, and, in the closed space environment, shifts in species diversity with unknown consequences. In fact, microgravity-induced changes in the growth and metabolism of fungi and bacteria have been observed in several experiments conducted aboard US and Russian spacecraft [5].

Current determinations of the susceptibility of plastics and other synthetics to fungal attack are based on standard challenge tests such as ASTM C665 [3] and MIL-STD-810 E [8]. The use of construction and finishing materials that fail these tests is limited on spacecraft. Data obtained from these relatively short-term tests, however, may not be suitable for predicting the resistance of materials to be exposed in an atypical environment for several decades. We report here the use of more stringent testing procedures to determine the resistance to fungal colonization of fabrics and materials considered for prolonged use in space.

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MATERIALS AND METHODS

Swatches of fabric and pieces of other materials (Table 1), 1.0–2.0 cm^2 in size, were pierced with thin stainless steel wire and fastened to stainless steel grills designed for use when barbecuing foods. In these processing steps, all materials were handled with gloves, and all test materials were manipulated using aseptic technique with sterile forceps under a laminar flow hood. Test materials such as elastic cords that were not amenable to piercing with the wire were cut into segments of about 5 cm and fastened to the grills with wire. The grills were suspended from the hood of an acrylic-domed, thermoplastic chamber that contained trays of nonsterile potting soil positioned over a water reservoir (Fig. 1). The chamber was equipped with a fan and its temperature maintained between 25 °C and 32 °C and its relative humidity over 95%. Before the test was begun, more than 50 species of fungi and numerous bacteria were isolated from the chamber; air samples from the chamber collected with a single stage Andersen air sampler (Atlanta, GA, USA) indicated that the number of fungal propagules exceeded 14 000 CFU per cubic meter of air. During the test period, various materials not related to this study were added to or removed from the chamber. Such materials included painted metals, gypsum wall board, synthetic carpets

TABLE 1

Fungal colonization of materials (space station)[a]

Code	Material	Predominant fungi	
		Initial culture[b]	After 28 days
1	Aplix nylon (blue velcro)	negative	*Acremonium obclavatum*[d]
2	Green webbing type II GI 888	*Aspergillus niger*	*Acremonium obclavatum*[d]
		Penicillium sp.	*Asp. flavus*[c]
4	Teflon, FEP St 92T 837-05	negative	*A. obclavatum*[d]
5	Neoprine	*Penicillium* sp.	negative for fungi
6	Elastic cord 8305-00-3116	negative	*Aspergillus flavus*[d]
			A. obclavatum[c]
7	Nomex fabric w/adhesive	*Asp. niger*	*A. obclavatum*[c]
8	Spandex	*Penicillium* sp.	*Asp. flavus*[d]
10	Mylar	negative	*A. obclavatum*[c]
11	Urethane-coated nylon Type 1 MIL-C-83489	negative	*Pseudomonas* sp.[e]
12	Nomex fabric	*Asp. niger*	*A. obclavatum*[c]
13	Durette X400-11 (insulation, soundproofing)	*Bacillus* sp.	*Bacillus* sp.
14	Aplix, white	negative	*A. obclavatum*[c]
15	Pyrell	*Asp. niger*	*Asp. flavus*[c]
16	White minicell foam L200FR	negative	*Asp. flavus*[c]
			Penicillium sp.[c]
17	Temper (blue foam)	negative	*A. obclavatum*[d]
18	Solimide (yellow foam)	negative	*Asp. japonicum*
19	Chemglass PREM 14MIL	negative	*A. obclavatum*[d]
			Asp. flavus[c]
20	Fluroglas beta cloth x-3897	negative	*A. obclavatum*[d]
21	Elastic SS 1100	*Asp. niger*	*A. obclavatum*[c]
22	Nylon velcro	negative	*Penicillium funiculosum*[c]
24	Polyimide	*Asp. niger*	*A. obclavatum*[d]
25	Cotton, blue	*Asp. niger*	*Penicillium funiculosum*[d]
		Penicillium sp.	*Asp. fumigatus*[c]
26	Nylon-coated polyethylene film	negative	*A. obclavatum*[c]
27	Ortho fabric	negative	*Asp. flavus*[c]
			A. obclavatum[c]
			Acremonium sp.[c]
28	Zitex A	negative	*A. obclavatum*[c]

[a] Incubated in test chamber with >95% relative humidity, 25–32 °C.
[b] Positive cultures with no more than one or two colonies (except for #13).
[c] Extensive colonization of at least one imprint area.
[d] Microscopic evidence of colonization; hyphae with conidiophores and conidia.
[e] Apparent colonization by bacteria.

(untreated or treated with antimicrobial agents), fiberglass insulation, and white cotton and white birch test strips [4,7].

Before test materials were placed in the chamber, control sections of the materials were imprinted (both sides) onto Mycological agar (Difco Laboratories, Detroit, MI, USA) containing 0.05% chloramphenicol, and the test sample was placed on a separate section of the plate. This procedure was repeated weekly for at least four weeks with all test materials removed from the chamber. At each sampling period, a section of each test material was also removed for microscopic observations. Control samples of each were also placed into chambers (2.3-L sealed glass jars or 25-L polycarbonate vessels) without soil [4]. These samples were incubated as described above for up to 12 months.

Samples for microscopic observation were stripped with clear acetate or polyvinyl tape and stained with lacto-phenol cotton blue for light microscopy or prepared for scanning electron microscopy as described previously [1]. Colonization was termed 'microscopic' if hyphae with mature conidiophores and conidia or other reproductive structures were observed under magnification. Colonization was termed 'macroscopic' if fungal growth could be observed without magnification. Representative isolates of fungi were subcultured on various enriched agars for identification by standard procedures [2].

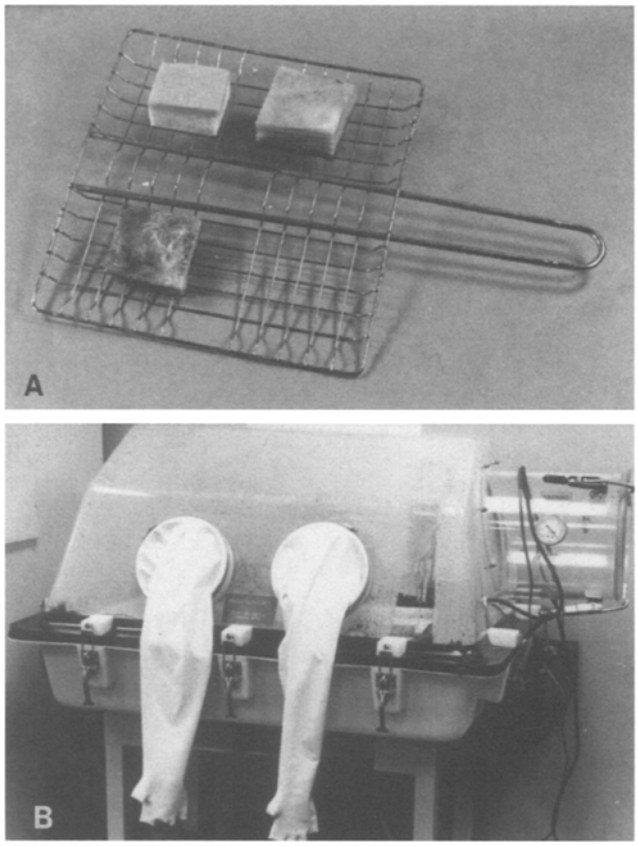

Fig. 1. (A) Fiberglass insulation sections on stainless steel grill that was suspended from roof of challenge chamber. (B) Acrylic-domed test chamber.

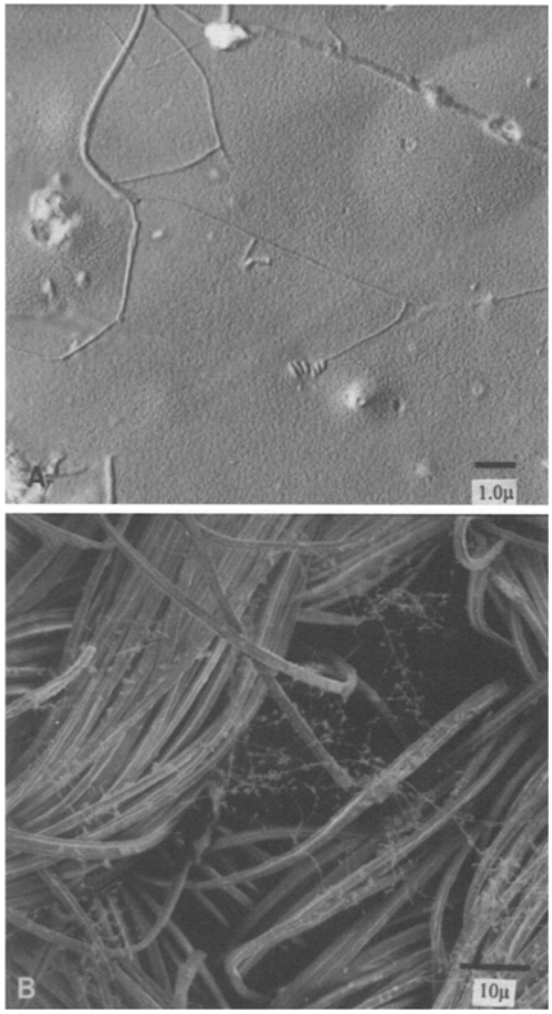

Fig. 2. (A) Acetate tape mount from Tempra foam (test material 17) showing hyphae and conidiophore with conidia of *Acremonium obclavatum* after 21 days in the test chamber. (B) After 28 days, hyphae and conidiophores are enveloping fibers of polyimide fabric (test material 24) (SEM).

RESULTS

Initial cultures of most test materials (i.e. before their placement in the test chamber) were negative for fungi; however, an insulation and soundproofing material (Code 13) yielded profuse colonies of a *Bacillus* sp. When initial cultures were positive for fungi, usually only one or two colonies of *Aspergillus niger* or a *Penicillium* sp. (subgenus *Furcatum*) were observed (Table 1). Within 28 days in the chamber, most fabrics and other types of materials were positive on culture for *Acremonium obclavatum* and *Aspergillus flavus*. Microscopic colonization of these test materials frequently was extensive, covering all surfaces (Fig. 2). This colonization, however, was not detected by unaided vision on any of the test materials within the 28-day test period. In contrast, other test materials, (e.g. cellulose-faced gypsum wall board, white cotton and birch strips) exposed for the same period within the chamber, showed obvious macroscopic colonization and product degradation by fungi. Between 28 and 63 days in the test chamber, fungal colonization of a few synthetics became obvious to the unaided eye (Fig. 3). The density of bacterial colonization on test materials 11 and 13 increased for *Pseudomonas* and *Bacillus* spp., respectively, over the 28-day period, but few fungal colonies were obtained. Microscopic colonization of the insulation material (Code 13) by *A. obclavatum* occurred only after 56 days, when the bacterial population was noted to decrease. Only materials 13 and 18 demonstrated sporadic areas of microscopic colonization by *Aspergillus* spp. in the control chamber by 12 months.

DISCUSSION

Both synthetic and natural polymers (Code 25) were found to be susceptible to fungal colonization. The colonization was not obvious with unaided vision (as was the case for materials such as the white cotton test strips or the cellulose facing of gypsum wall board), nor was it readily detectable under 40 × magnification. Extensive mycelia with conidiophores and conidia were observed under higher magnifications. Three fungi, *Penicillium funiculosum, Aspergillus flavus*, and *A. niger*, all of which are recommended as challenge organisms in standard challenge procedures (MIL-STD-810E or ASTM C665), were isolated from the test samples. The other two fungi used in these procedures, *Aspergillus versicolor* and *Chaetomium globosum*, colonized other materials in the chamber but were not

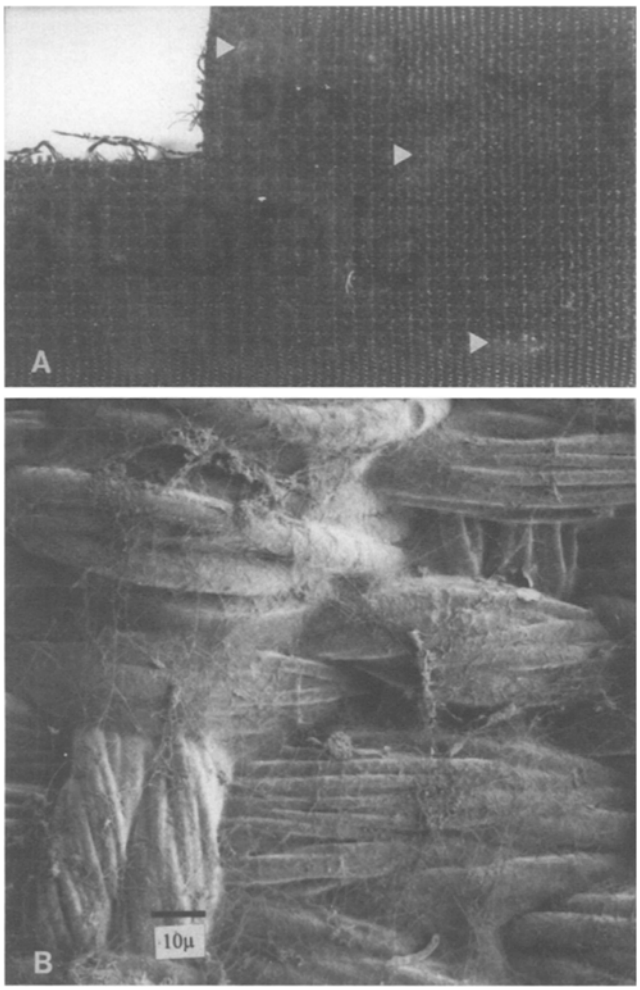

Fig. 3. (A) Velcro® nylon (test material 1) with areas of 'macroscopic' fungal colonization (arrows) after 63 days in the test chamber. (B) Low magnification SEM of hyphae enveloping the same material at day 63.

isolated from the test samples. According to several standard procedures, if fungal growth were not obvious at the end of a 28-day test period, or if growth were less than that on a control cellulosic substrate, the test material would 'pass', i.e. would have resisted fungal attack. In the MIL 810E test, the test duration may be extended to 84 days when a greater degree of certainty (less risk) is required in determining the existence or effect of fungal growth. Even with extended test durations, however, most if not all of our test materials could have been considered to resist fungal attack when evaluated by most standard approaches designed mainly with concerns on biodegradation. Our results indicate that certain standard fungal-challenge procedures are inadequate for many noncellulosic substrates that are expected to resist fungal colonization over extended periods.

It may be argued that our tests were performed under unrealistically high humidity conditions and potential soiling but it is important to note that space craft interiors with overall low humidity invariably have micro foci of high humidity, especially crevices and overlapping areas adjacent to water or moisture sources. Interior surfaces in space craft or in buildings are exposed also to volatiles from off-gassing of a variety of materials. Fungi have been known to produce extensive colonizations in space craft during flights [9]. The differential adsorption or absorption of organics may affect markedly the growth of fungi on recalcitrant substrates. Our chamber contained a fluctuating myriad of volatile organics (unpublished data) that enhanced the rate and extent of fungal growth on normally recalcitrant substrates. Volatiles from soil were noted previously to enhance fungal colonization of fiberglass insulation materials [4]. Fungal colonizations in a closed environment may exacerbate allergenic and toxigenic problems and may eventually result in material failures. Identification of interior substrates in space craft that are prone to absorb/adsorb various volatiles and support fungal growth may be critical to the success of future long term missions.

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