

Comparison of Media for Detection of Fungi on Spacecraft

C. M. HERRING, J. W. BRANDSBERG, G. S. OXBORROW, AND J. R. PULEO

Environmental Microbiology Section, Ecological Investigations Program, Center for Disease Control, Cape Canaveral, Florida 32920

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Five media, including Trypticase soy agar (TSA; BBL) pour plates, spread plates of TSA, Mycophil agar with chloromycetin, Mycophil agar with chloromycetin and Actidione, and cornmeal agar with chloromycetin were quantitatively and qualitatively compared for the detection of fungi on spacecraft. Cornmeal agar with chloromycetin yielded the highest number of fungal colonies, although not always significantly higher than Mycophil agar with chloromycetin or TSA spread plates. Cornmeal agar with chloromycetin also gave the best qualitative representation of fungi on the spacecraft, recovering 68% of the genera found from all media. This medium yielded 10 times the number of fungal colonies and 3 times the number of genera found on TSA pour plates as currently used for spacecraft assay.

The procedures for the microbiological assay of space hardware call for the exclusive use of Trypticase soy agar (TSA) recovery medium and incubation at 32 C (4). These procedures limit the assay to detection of heterotrophic, mesophilic microorganisms, which make up the majority of microorganisms found on spacecraft surfaces. This is especially true in controlled environments where man is the chief source of contamination (3). Soil microorganisms (bacterial sporeformers, fungi, and actinomycetes) can, however, make up a small but significant portion of the microbial load in areas where there are few environmental controls.

Numerous other culture media have been evaluated for the routine assay of space hardware; most were bacteriological media, and, although some improvement was seen for the detection of certain groups of bacteria, no one medium was so superior to TSA that it was considered for routine use (2). Mycophil agar and TSA were used for the detection of fungi from the Apollo 10 and 11 spacecraft samples. Although the results were not conclusive, it appears that there were no differences in the number of fungal colonies observed between the two media (6).

Although fungi were detected on Apollo spacecraft, they posed no real problems because they were not detected in large numbers on the interior surfaces of the spacecraft, the flights were of relatively short duration (ca. 14 days), and the environment in the cabin did not seem conducive to their growth. With the advent of longer duration spaceflights and more complex

environments such as the Skylab program, fungi could present a new problem, and their adequate detection on space hardware becomes important. These spacecraft will likely contain more fungi due to their size and complex interior configuration. The crews will man these craft for long periods and there will likely be areas, such as food chillers, where fungi may be able to grow readily.

The object of this study was to compare five media, including TSA pour plates, for the detection of fungi on space hardware.

MATERIALS AND METHODS

The five media and methods compared were TSA (BBL) pour plates, TSA spread plates, Mycophil agar (BBL) spread plates with 50 μ g of chloromycetin per ml (chloramphenicol, Parke-Davis & Co.) added, Mycophil agar spread plates with 50 μ g of chloromycetin per ml and 500 μ g of Actidione per ml (cycloheximide, Upjohn Co.) added, and cornmeal agar (BBL) spread plates with 50 μ g of chloromycetin per ml added. The TSA pour plates are the standard method currently used to assay spacecraft hardware for total microbial contamination (4). TSA spread plates were used to provide a comparison of surface growth versus submerged growth. Both Mycophil and cornmeal agars are media commonly used for the cultivation of fungi. Chloromycetin was incorporated into these two media as a broad-spectrum bacterial inhibitor to prevent inhibition of fungal colonies by bacteria. Actidione inhibits many fungi, and it was incorporated into Mycophil agar along with chloromycetin to facilitate detection of slow-growing fungi.

The instrument unit (IU) and the Saturn S4B stage (S4B) of the Apollo 16 spacecraft were chosen as the sampling areas. In previous samplings of Apollo

spacecraft for total microbial contamination, these two modules yielded the highest number of fungi (6). Fifteen sites in each module were sampled twice with 1 week between samplings.

Sterile cotton swabs moistened in sterile distilled water were rubbed over the surface to be sampled (4 in²). Each swab was returned to a sterile screw-cap test tube (16 by 150 mm) containing 5 ml of sterile buffered rinse solution with 0.02% (vol/vol) polyoxyethylene sorbitan monooleate (Tween 80 source) (4). The swab heads were broken off below the portion of the handles touched by the sampler. Tubes were immediately taken to the laboratory, agitated on a Vortex mixer for 5 to 10 s, placed in an ultrasonic tank (Branson Instruments, Inc., Stamford, Conn.) containing a 0.3% Tween 80 solution, and sonicated for 2 min at 25 kHz (5).

After sonication, duplicate 1-ml portions from each tube were plated as pour plates with TSA. Duplicate 0.2-ml portions were spread on the surface of TSA plates, Mycophil with chloromycetin, Mycophil with chloromycetin and Actidione, and cornmeal with chloromycetin plates. The TSA pour plates were incubated at 32 C. All other plates were incubated at room temperature (25 C).

Counts of the fungal colonies were made after 7 days. Some plates were held up to 21 days to allow for the development of slow-growing colonies. Colonies were picked at various times after 7 days and up to 21 days, depending on the amount of growth on the plate. All colony types observed on each medium were either picked and placed on cornmeal agar slants or identified on the plate. A total of 298 colonies were isolated and identified, and an additional 124 were identified on the plates, making the total number identified 422.

Many of the fungi were identified by the Mycology Section, Center for Disease Control, Kansas City, Kan.

At least three media were used for identification of all fungi. These included czapek agar (Difco), malt agar (7), and cornmeal agar (1). Isolates were planted on each of the media by using a modified cover-slip culture technique. Blocks of agar were cut from the center of a plate and placed around the periphery of the medium. One or more sides of the blocks were inoculated with spores or mycelium, or both, of a particular isolate. Cover slips were removed from a container of 95% ethanol, flamed, and then placed on each block. After sufficient growth was present on the cover slips so that adequate sporulation had occurred, the cover slips were removed, placed over a drop of lactophenol-cotton blue on a slide, and examined microscopically.

Ascomycetes and pycnidial forms were normally identified, from structures on the surface of one or more media, after they had grown down off the block and sporulated. All plates were incubated at room temperature (25 C) and subjected to ordinary diurnal variation. Most fungi were identified only to genus, and standard references were used in making determinations. When necessary, other media such as potato dextrose agar were used.

RESULTS

The average number of fungal colonies per milliliter of sample on each type of medium is shown in Table 1. Cornmeal agar with chloromycetin grew the largest number of fungal colonies from both modules. Mycophil with chloromycetin was next most productive, followed by TSA spread plates, TSA pour plates, and Mycophil with chloromycetin and Actidione. *t*-Tests were performed comparing each medium, except Mycophil with chloromycetin and Actidione, to see if these data represent significant differences in the number of fungal colonies obtained from the two modules (Table 2). The comparisons revealed that TSA spread plates, Mycophil with chloromycetin, and cornmeal with chloromycetin yielded significantly higher colony counts from both modules than did TSA pour plates. There were no statistically significant differences in results obtained when cornmeal agar with chloromycetin was compared with Mycophil plus chloromycetin or TSA spread plates with Mycophil plus chloromycetin. On the IU, significantly higher fungal colonies were observed on cornmeal agar with

TABLE 1. Number of fungal colonies per milliliter of sample on various media^a

Determination	TSA pour	TSA spread	Mycophil with chloromycetin	Cornmeal with chloromycetin	Mycophil with chloromycetin Actidione
IU	0.6	1.4	2.3	3.1	0.1
S4B	0.8	8.0	10.3	11.1	0.3

^a Average number from 30 plates.

TABLE 2. Comparisons of media for significant differences in the enumeration of fungal colonies^a

Media	TSA spread		Mycophil with chloromycetin		Cornmeal with chloromycetin	
	IU	S4B	IU	S4B	IU	S4B
TSA pour	No	Yes ^b	Yes	Yes	Yes	Yes
TSA spread			No	No	Yes	No
Mycophil with chloromycetin					No	No

^a *P* < 0.05.

^b *P* < 0.10.

chloromycetin than on TSA spread plates. On the S4B, however, there was no difference.

Fifty different fungi were isolated from all media from both modules. A comparison of the number of genera observed on each medium is presented in Table 3. Eleven genera (22% of total) were isolated from TSA pour plates. Twenty-three and 27 genera (46 and 54% of the total) were isolated from TSA spread plates and Mycophil agars, respectively. The highest number of genera, 34 (68%), were isolated from cornmeal agar with chloromycetin. Five genera (10%) were isolated from Mycophil agar with chloromycetin and Actidione; however, none of the "slow-growing" types grew on it even though these were the species we had hoped to isolate from these media.

Thirty-five *Aspergillus* isolates were keyed to one of eight species: *A. niger*, *A. versicolor*, *A. fumigatus*, *A. flavus*, *A. terreus*, *A. ustus*, *A. deflektus*, and *A. tamarii*.

A wide variety of fungi representing four classes was found on the spacecraft: *Phycomycetes*, *Ascomycetes*, *Basidiomycetes*, and *Fungi imperfecti*.

DISCUSSION

The quantitative comparisons of the media showed that TSA pour plates, as used for the total microbial assay of space hardware, yielded significantly fewer fungi than any of the other three media to which it was compared. Because TSA spread plates yielded significantly more fungal colonies than TSA pour plates, one can conclude that, since most fungi are aerobic, the spread plate technique is better suited for the enumeration of these fungi. Although a greater number of colonies were obtained from the IU samples on cornmeal agar, we did not find significantly more colonies on cornmeal and Mycophil agar than on TSA spread plates in other cases. These two media contained chloromycetin, which inhibited bacterial growth, an advantage that made colony counting and isolation much easier. TSA with chloromycetin was not tested, but this combination might possibly be useful for the detection of fungi. Cornmeal agar with chloromycetin yielded the greatest number of fungal colonies from both the IU and S4B.

Qualitative comparisons of the media showed the importance of surface growth. Twice the number of genera were isolated from TSA spread plates as from TSA pour plates. Mycophil agar with chloromycetin and TSA spread plates were about equally efficacious regarding the number of genera isolated on each, but

TABLE 3. *Genera and types of fungi isolated on various media*

Genera or type	TSA pour	TSA spread	Mycophil C ^a	Cornmeal C ^b	Mycophil C&A ^c
<i>Curvularia</i>	+	+	+	+	
<i>Penicillium</i>	+	+	+	+	
<i>Aspergillus</i>	+	+	+	+	
<i>Spegazzinia</i>	+	+	+	+	
<i>Trichoderma</i>	+	+			
<i>Chaetomium</i>	+	+	+	+	
<i>Thielavia</i>	+	+			
<i>Fusarium</i>	+	+		+	
<i>Bipolaris</i>	+	+	+	+	
<i>Monocillium</i>	+				
<i>Rhizopus</i>	+	+	+	+	
<i>Cladosporium</i>		+	+	+	+
<i>Asperisporium</i>		+			
<i>Periconiella</i>		+	+	+	
<i>Paecilomyces</i>		+	+	+	
<i>Nigrospora</i>		+	+	+	
<i>Sphaeropsis</i>		+			
<i>Pucciniopsis</i>		+	+		
<i>Mucor</i>		+	+	+	+
<i>Chaetophoma</i>		+			
<i>Pestalotia</i>		+	+	+	
<i>Pithomyces</i>		+	+	+	
<i>Rhinocladium</i>		+			
<i>Phoma</i>			+	+	
<i>Geotrichum</i>			+		
<i>Piggotia</i>			+	+	
<i>Torula</i>			+		
<i>Cytospora</i>			+		
<i>Helminthosporium</i>			+		
<i>Leptosphaeria</i>			+		
<i>Cephalosporium</i>			+	+	+
<i>Chaetomella</i>			+		
<i>Aureobasidium</i>			+	+	
<i>Calcarisporium</i>				+	
<i>Humicola</i>				+	
<i>Oidiodendron</i>				+	
<i>Alternaria</i>				+	
<i>Monodictis</i>				+	
<i>Chitonospora</i>				+	
<i>Pseudobotrytis</i>				+	
<i>Mammaria</i>				+	
<i>Coniochaeta</i>				+	
<i>Papularia</i>				+	
<i>Epicoccum</i>				+	
<i>Phialophora</i>				+	
<i>Scopulariopsis</i>					+
<i>Phycomycete</i>				+	
<i>Basidiomycete</i>				+	
<i>Sterile mycelium</i>		+	+	+	+
Total	11	23	27	34	5
Percent of total	22	46	54	68	10

^a Mycophil with chloromycetin.

^b Cornmeal with chloromycetin.

^c Mycophil with chloromycetin and Actidione.

cornmeal with chloromycetin seemed to give the best representation of the fungi on the spacecraft, recovering 68% of the genera found on all media. Only one genus not found on other media was recovered from Mycophil agar with

chloromycetin and Actidione. This was *Scopulariopsis*, which is not considered a "slow-growing" organism.

The majority of the genera isolated and identified were common saprophytic fungi, found in the soil and on decaying organic matter, and common plant pathogens. Because of their ability to degrade many materials, many of these fungi, although harmless to man, could cause problems in a Skylab-type environment. These organisms can be expected to proliferate on nearly any substrate in areas of high humidity (above 65%). Some of these saprobes, such as *Aspergillus*, *Mucor*, and *Rhizopus*, are recognized as "opportunistic pathogens." Of the eight species of *Aspergillus* identified, four are recognized as potential pathogens. *A. fumigatus* is the most frequent cause of pulmonary aspergillosis, with *A. flavus* occurring less frequently. *A. niger* is the usual cause of otomycosis or fungus infection of the ear, and *A. terreus* causes cellulitis and subcutaneous granulomata (1). These opportunistic pathogens are not generally harmful to healthy individuals, but, during chemotherapy, periods of stress, or in the presence of predisposing infections, they can invade the body and be extremely hazardous, especially when spores are inhaled in large quantities. Also, inhalation of large numbers of *A. fumigatus* spores may cause disease without predisposing factors (8). The spores of many nonpathogenic fungi may cause allergic reactions after repeated or prolonged exposure.

The combination of qualitative and quantitative comparisons of the media tested show cornmeal with chloromycetin to be the medium of choice for the sampling of fungi on spacecraft hardware. This medium yielded the highest counts and the greatest number of genera of fungi. From the combined data of both modules, cornmeal with chloromycetin yielded 10 times the number of fungal colonies and 3 times the number of genera found on TSA pour plates as currently used for spacecraft assay.

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