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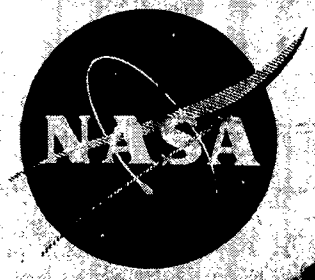
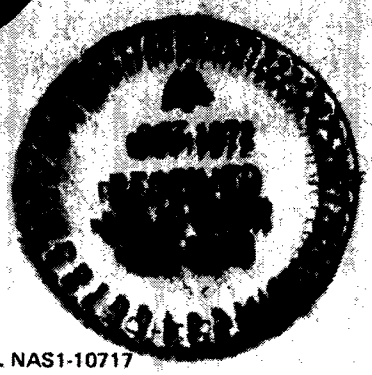
FEBRUARY 1972





Prepared Under Contract No. NAS1-10717

By

Biotechnology and Power Department
McDonnell Douglas Astronautics Company
Huntington Beach, California

For
LANGLEY RESEARCH CENTER
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

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ANALYSIS OF FUNGAL-TYPE ISOLATES TAKEN FROM A 90-DAY MANNED TEST OF AN ADVANCED REGENERATIVE LIFE SUPPORT SYSTEM

FEBRUARY 1972

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National Aeronautics and Space Administration

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TABLES

SUMMARY

Fungal-like cultures isolated before, during, and after the 90-day test from samples of Space Station Simulator (SSS) atmosphere, surfaces, subsystem components and crew dermal sites were identified to genus. Out of the original 525 isolates, approximately 80% were classified as bacteria. Laboratory methods (culture media, moisturization, and incubation temperatures) favored the recovery of medically significant bacteria rather than fungi. Therefore, fungal isolates were mostly hardy, non-fastidious types which are ubiquitous in soil and air and commonly contaminate laboratory cultures of pathogens. Predominant isolates were species of Aspergillus, Penicillium, Pullularia, Rhodotorula, and various yeasts. No instances of fungal proliferation were observed; test data reflect the "survival" of environmental types indigenous to the SSS pretest.

INTRODUCTION

The 90-Day Test provided an unusual opportunity to assess potentially detrimental changes in human and environmental microflora during an extended simulation study in a microbiologically closed system. During the test, numerous fungal-type isolates were recovered from the atmosphere, surfaces, subsystem components, and crew dermal sites, but were not identified. This study was instituted to 1) yield more specific conclusions concerning fungal die-off and/or buildup, transmission mechanisms, and materials deterioration and 2) supplement microbiological findings presented in the 90-Day Test final report (Reference 1).

SECTION I

ISOLATION AND MAINTENANCE OF ISOLATES

Sampling, passout, and laboratory processing procedures are outlined in Tables 1 and 2. Sabouraud Dextrose Agar (SDA) was the primary isolation medium for fungi from the crew dermal sites (axilla, perineum and toe web), station surfaces in the food management and hygiene areas, atmosphere, and post-test subsystem surfaces and components.

Different fungal-like colonies were picked and streaked onto fresh SDA plates for purification. Well-isolated colonies were each transferred to a SDA slant. Slants were incubated at the same temperature as the isolation plates. The 525 slants which resulted from this procedure during and after the test were stored at 4-6°C.

For culture maintenance, subcultures of all isolates were periodically made onto fresh SDA slants (ca. every 2 months). Not all the transfers were successful due to loss in viability. After 4 months' storage at 4-6°C, the original slants were placed at room temperature after addition of sterile mineral oil.

TABLE 1

SUMMARY OF SAMPLING PROCEDURES

SAMPLE	LOCATION OR TYPE	METHOD	SCHEDULE	
			SAMPLING	PASSOUT
CREW	AXILLA, PERINEUM, AND TOE WEB	COTTON SWAB MOISTENED WITH PHYSIOLOGICAL SALINE	WEEKLY	WEEKLY
SURFACES	IOCM X IOCM AREAS: IN FOOD MANAGEMENT AND HYGIENE COMPARTMENTS	COTTON SWABS MOISTENED WITH CASEIN SOY BROTH. SURFACE CLEANED WITH 1:750 ZEPHIRAN AFTER PASSOUT	WEEKLY	WEEKLY
	5 CM X 5 CM AREAS: SPIROMETER INLET, COMMODE SEAT, FLOOR UNDER BUNKS, RECREATION TABLE TOP, MICROWAVE OVEN, ETC.	COTTON SWABS MOISTENED WITH CASEIN SOY BROTH	POST-TEST	NONE
AIR	EQUIPMENT (FORWARD) AND CREW (AFT) COMPARTMENTS	REYNIERS SAMPLERS RUN FOR 1 HR AT 1 FT^3 OF AIR PER MIN. CASEIN SOY AGAR PLATES INCUBATED ON-BOARD AT 35°C FOR 24 HRS	EVERY 2 WEEKS. DAY BEFORE PASSOUT	EVERY 2 WEEKS
HARDWARE SUBSYSTEM COMPONENTS	ION-EXCHANGE RESINS, CHARCOAL, FILTERS, GARBAGE, FOOD TRAYS, ETC.	IMMERSION OF SMALL PORTION OF MATERIAL IN CASEIN SOY BROTH	POST-TEST	NONE

TABLE 2

PRIMARY CULTURAL CONDITIONS FOR RECOVERY OF FUNGI

SAMPLE	*CULTURE MEDIUM	INCUBATION
AXILLA, PERINEUM AND TOE WEB	[1]SABOURAUD DEXTROSE AGAR	37°C FOR 24 HRS OR 22°C FOR 7 DAYS
SURFACES	[1]SABOURAUD DEXTROSE AGAR	37°C FOR 24 HRS OR 22°C FOR 7 DAYS
AIR	[2]CASEIN SOY AGAR	ONBOARD LAB: 35°C FOR 24 HRS [3]OUTSIDE LAB: 37°C FOR 24 HRS + 22°C FOR 7 DAYS
POST-TEST HARDWARE COMPONENT SURFACES	[1]SABOURAUD DEXTROSE AGAR	37°C FOR 5 DAYS 22°C FOR 5-7 DAYS
POST-TEST HARDWARE SUBSYSTEM COMPONENTS	[4]CASEIN SOY BROTH ↓ SABOURAUD DEXTROSE AGAR	37°C FOR 24 HRS 37°C FOR 5 DAYS 22°C FOR 5-7 DAYS

NOTES:

1. SWAB SAMPLES USED TO STREAK AGAR PLATES.
2. PLATES EXPOSED TO ATMOSPHERE IN ON-BOARD REYNIERS AIR SAMPLERS.
3. BACTERIAL COUNTS MADE AFTER 24 HRS @ 37°C AND FUNGAL COUNTS AFTER 7 DAYS @ 22°C.
4. SAMPLE PORTIONS IMMERSED IN BROTH TUBES. AFTER INCUBATION PERIOD, PLATES WERE INOCULATED FROM THE BROTH CULTURES FOR COLONIAL ISOLATION.

* MEDIA FROM HYLAND, DIVISION OF TRAVENOL LABORATORIES, INC., COSTA MESA, CALIFORNIA.

SECTION II

SCREENING CULTURES BY GROSS MORPHOLOGY AND MICROSCOPIC EXAMINATION

Prior to oil coverage, all original slants were examined and grouped by gross similarities in pigmentation, surface texture, type of growth (vegetative or reproductive stages), presence of liquid droplets, etc. Twenty-six (26) groups or "gross morphological types" were designated. Also before addition of the oil, smears were prepared and stained for Gram reaction. Microscopic examination resulted in preliminary differentiation of molds and yeasts from bacteria. Table 3 shows the results of the screening procedures.

TABLE 3

PRELIMINARY SCREENING OF FUNGI FROM SABOURAUD DEXTROSE AGAR ISOLATES BY GROSS MORPHOLOGICAL GROUPING AND MICROSCOPIC EXAMINATION

Gross Morph. Type No.	* Morph. Type Description	** Microspopic Screening Results No. Isolates/Morph. Type	
		Before Exam.	After Exam.
1	Pumpkin, moist	1	0
2	Tan, with olive-green specks, moist	1	0
3	Black mycelium and white sporulation	1	1
4	Lemon-green with dull sheen	1	1
5	Tan mycelium and sporulation with corrugated surface	1	1
6	Black mycelium and sporulation	1	1
7	Tan → black, corrugated	2	2
8	Yellow-cream, dry	1	0
9	Yellow → blue-green sporulation	1	1
10	Cream-colored with purple specks, moist	1	0
11	Cream → cocoa, dry and wrinkled	9	4
12	Yellow-orange, moist	10	1
13	Beige mycelium and white sporulation - dry	4	4
14	Salmon pink → black sporulation	5	5
15	Black mycelium with olive-green sporulation	2	2
16	Tan sporulation, moist and corrugated	1	1
17	Black mycelium with grey-white sporulation	1	1
18	Salmon pink, dry and corrugated	2	2
19	Salmon pink, moist	15	13
20	Blue-green mycelium and white sporulation	14	14
21	Tan → brown mycelium - no sporulation	7	7
22	Yellow mycelium and olive-green sporulation	20	20
23	Cream → beige, very moist surface	33	2
24	Yellow-cream, moist	53	22
25	Cream-colored, dry	155	50
26	Cream-colored, very moist	183	38

* Growth on Sabouraud Dextrose Agar slant

** After microscopic examination of Gram-stained smears, 193 out of the original 525 slant cultures were presumptively identified as fungi.

SECTION III

GENERIC CLASSIFICATION OF FUNGAL-LIKE ISOLATES

Classification to genus of all 193 isolates which were presumptively identified as fungi was performed at the California State College at Long Beach mycology laboratory by Dr. F. E. Swatek, consultant. Original (under oil) and most recent transfer slant cultures (where available) were forwarded to Dr. Swatek in May 1971. The following is a description of his laboratory procedures:

If the original and most recent transfer slants did not macroscopically match, cultures were attempted from the original (under oil). Out of 35 cultures in this series, 11 did not grow, and 7 of the original slants, which were successfully subcultured at this time, did not agree with most recent transfer slants. Identification was based on the original slant culture in these cases. If viable cultures were unavailable, studies were directly made on original slants. Identification was difficult because the material was old (dating from the period of June-September 1970). In some instances, only a designation of

moniliaceous, i.e., colorless mycelium and spores absent, or dematiaceous, i.e., colored mycelium and spores absent, was made. Finally, the amount of material present in a few original slants was insufficient even to arrive at a basic designation; it appeared that these slant cultures contained only the original inoculum and were probably of bacterial origin.

Viable cultures were transferred to Potato Dextrose Agar (PDA) if they had a mycelial appearance and streaked on Blood or Trypticase Soy Agar media if they appeared bacterial or yeastlike. Lactophenol mounts were made of each subculture and microscopically examined. Penicillia and aspergilli were transferred to Czapek's Agar. Cultures which did not readily sporulate on PDA were transferred to appropriate media, as dictated by colonial morphology--these included Cornmeal, Carnation, Czapek's, and Moyer's Multiple Threat Agar media. In addition, some slide cultures (using SDA and PDA) were prepared in special cases. Incubation temperatures for fungal cultures ranged between 28° and 32°C.

For all colonies appearing to be bacteria, smears were prepared and stained for Gram reaction and spores. Characterization was based on general morphology, Gram reaction, and the presence or absence of spores.

Colonies which appeared to be yeasts were microscopically studied using lactophenol mounts and Gram stained smears. Transfers were made to PDA plates for purity checking and cell morphology study. After purification, the following procedures/tests were performed.

1) Ascospore development was encouraged by the use of plaster of Paris blocks, V-8 Juice Agar, and wedges of carrot, potato and cucumber.

2) Fermentation tests were conducted in accordance with the procedure of Wickerham (Reference 2). Sugars were added to a Yeast Extract--Peptone Base as sole sources of carbon, viz., glucose, galactose, sucrose, maltose, lactose, melibiose, and inositol (all in 2% final concentration) and raffinose (4%).

3) Sugar assimilation tests were performed using Yeast-Nitrogen Base and the same group of sugars as above plus xylose.

4) Nitrite and nitrate assimilation tests were conducted in liquid media using Yeast-Carbon Base.

5) Ethylamine hydrochloride utilization was tested for all samples.

6) Growth or no growth was ascertained on Vitamin-Free Agar medium.

Note: All media were either obtained from Difco Laboratories, Detroit, Michigan, or specially formulated at the Long Beach State College laboratory.

Classification of yeasts was based on the second edition of Lodder (Reference 3). Identification of 7 yeast cultures is still incomplete pending manifestation of sporulation stages.

SECTION IV

RESULTS

After the identification to genus of fungal isolates selected during the 90-Day Test was completed, data were analyzed for quantitative and qualitative changes in types indigenous to the crew, their environment, and life support systems. Possible occurrences of cross infection, build-up and equipment deterioration were especially investigated. Table 4 presents a summary of taxonomic results.

TABLE 4

SUMMARY OF FUNGAL IDENTIFICATION

GENUS OR TYPE	SAMPLE GROUP PERCENTAGE OF ISOLATES/GENUS OR TYPE					
	DERMAL *(90)	REYNIERS (28)	STATION SURFACE (14)	POST-TEST SURFACE (44)	POST-TEST STORED WASTE (5)	POST-TEST COMPONENT (12)
ASPERGILLUS	12	25	7	2		
CANDIDA		4				
CEPHALOSPORIUM			7			
CRYPTOCOCCUS				7		
ERODIUM				2		
GEOTRICHUM (?)			7			
HORMODENDRUM	2	4				
PENICILLIUM	2	25	14	27		
PULLULARIA			29	9		
RHODOTORULA	4	11	7	7		8
SCHIZOSACCHAROMYCES					40	
STACHYBOTRYS	1					
TORULOPSIS	7			5		8
Fungus-Dematiaceous Mycelium	2			2		
Fungus-Moniliaceous Mycelium		4				
Bacteria	64	20	29	37	60	76
Yeasts, Unidentified	3	7		2		8
Culture Irretrievable	3					

*NUMBER IN PARENTHESES = NUMBER OF ISOLATES/SAMPLE GROUP

Dermal Swabs

The recovery of fungi from crew dermal sites (axilla, perineum and toe web) is summarized in Tables 5 and 6. Most dermal isolates were types commonly found in the environment: species of Aspergillus, Hormodendrum, Penicillium and yeasts like Rhodotorula and Torulopsis. An unusual isolate was Stachybotrys, from Crewman #1's perineum on test day 4. This organism is commonly found in mucilaginous materials like packaging and not on human skin; it was probably introduced by some material used in personal hygiene. Rhodotorula and Torulopsis were consistently isolated from Crewman #3's perineum and axilla. The fact that Rhodotorula was not isolated until test day 53 may reflect an activity which introduced this previously sequestered organism into the SSS around test mid-point. A possible source may have been a fresh change of clothing containing sizing or starch, in which Rhodotorula is commonly found. Finally, Rhodotorula may have been transferred to Crewman #2 from Crewman #3 (see test day 81). Torulopsis may have been transferred to Crewman #3's skin from fermenting or rotting food materials.

Surface Samples

Fungal isolates from SSS station surfaces during and after the test are listed in Tables 7 and 8. Isolates included common environmental types: species of Aspergillus, Cephalosporium, Geotrichum, Penicillium, Pullularia, and Rhodotorula. Pullularia, commonly found in sputum and air, was most frequently recovered from the hygiene area.

Fungal-type isolates were recovered from 21 out of the 35 surface areas sampled immediately after crew egress. The location of these surfaces is indicated in Figure 1. Identification results are presented in Tables 9 and 10. Types recovered were the same as organisms found on station surfaces (see above)

TABLE 5

IDENTIFICATION OF SABOURAUD DEXTROSE AGAR ISOLATES

DERMAL SWAB SAMPLES

	Isolate Code No.	Test Day	Crew man	Dermal Site			Gross Morph. Type	Classification
				Axilla	Perineum	Toe Web		
	H 05	-4	1			X	24	Penicillium
	H 08	-4	2	X			24	Bacterium
	H 10	-4	2		X		25	Bacterium
	H 12	-4	2		X		24	Bacterium
	H 20	-4	3	X			26	Bacterium
*	H 22	-4	5			X	11	Ustilago
*	H 24	-4	5			X	25	Bacterium
	H 29	-4	3		X		24	Bacterium
	H 30	-4	3		X		25	Bacterium
	H 33	-4	4			X	5	dematiaceous mycelium
	H 34	-4	4			X	24	Bacterium
*	H 37	-4	5		X		25	Bacterium
*	H 40	-4	6		X		25	Bacterium
*	H 43	-4	6			X	26	Bacterium
	H 46	4	1		X		25	Bacterium
	H 47	4	1		X		25	Bacterium
	H 48	4	1		X		3	Stachybotrys
	H 49	4	1			X	22	Aspergillus
	H 65	11	1		X		25	Bacterium
	H 68	11	1			X	22	Aspergillus
	H 69	11	2			X	24	Penicillium
	H 75	11	4	X			17	dematiaceous mycelium
	H 78	11	4			X	26	Bacterium
	H 82	18	1	X			26	Bacterium
	H 84	18	2	X			26	Bacterium
	H 85	18	2		X		24	culture irretrievable
	H 91	18	3		X		25	Bacterium
	H 97	25	1	X			25	Bacterium
	H 103	25	2	X			26	Bacterium
	H 107	25	2			X	24	Bacterium
	H 111	25	3	X			25	Bacterium
	H 135	32	1	X			25	Bacterium
	H 136	32	1		X		25	Bacterium
	H 138	32	1			X	24	Bacterium
	H 139	32	2	X			24	Bacterium
	H 140	32	2	X			25	Bacterium
	H 143	32	2			X	24	Bacterium
	H 144	32	4	X			25	Bacterium
	H 145	32	4	X			22	Aspergillus
	H 59	34	4			X	24	Bacterium
	H 61	34	4			X	24	Bacterium
	H 154	39	1	X			26	Bacterium
	H 155	39	1		X		26	Bacterium
	H 159	46	2			X	24	Bacterium
	H 161	46	2			X	24	Bacterium

TABLE 5

IDENTIFICATION OF SABOURAUD DEXTROSE AGAR ISOLATES

DERMAL SWAB SAMPLES (CONT.)

Isolate Code No.	Test Day	Crew man	Dermal Site			Gross Morph. Type	Classification
			Axilla	Perineum	Toe Web		
H 162	46	3	X			26	Bacterium
H 163	46	3	X			26	Bacterium
H 164	46	3		X		25	Bacterium
H 166	46	4	X			22	Aspergillus
H 170	46	4		X		24	Bacterium
H 171	46	4			X	24	Bacterium
H 172	46	4			X	26	Bacterium
H 182	53	1		X		25	Bacterium
H 183	53	1			X	25	Bacterium
H 184	53	1			X	20	Aspergillus
H 185	53	1			X	24	Bacterium
H 191	53	3		X		19	Rhodotorula
H 192	53	3		X		26	Torulopsis
H 195	53	4		X		25	Bacterium
H 196	53	4			X	25	Bacterium
H 197	53	4			X	20	Aspergillus
H 205	60	1		X		25	Bacterium
H 206	60	1			X	24	Bacterium
H 207	60	1			X	25	Bacterium
H 210	60	2		X		25	Bacterium
H 212	60	2			X	20	Aspergillus
H 222	60	4			X	25	Bacterium
H 223	60	4			X	25	Bacterium
H 229	67	1			X	22	Aspergillus
H 233	67	3		X		26	unidentified yeast
H 234	67	2			X	15	Hormodendrum
H 235	67	3		X		19	Rhodotorula
H 250	74	1	X			25	Bacterium
H 251	74	1	X			25	Bacterium
H 253	74	2			X	24	culture irretrievable
H 254	74	3	X			19	Rhodotorula
H 262	81	2			X	19	Rhodotorula
H 263	81	3	X			26	Torulopsis
H 264	81	3		X		26	Torulopsis
H 268	81	4			X	25	Bacterium
H 275	88	1			X	22	Aspergillus
H 276	88	1			X	12	culture irretrievable
H 280	88	4	X			22	Aspergillus
H 286	+18	1		X		26	Bacterium
H 288	+18	1			X	7	Hormodendrum
H 289	+18	1			X	13	Aspergillus
H 290	+18	2			X	26	unidentified yeast
H 291	+18	4		X		26	Bacterium
H 292	+18	4			X	26	Torulopsis
H 293	+18	4			X	25	unidentified yeast

* These isolates were recovered from backup crewmen, who were not among the final 4 crewmen.

TABLE 6

RECOVERY OF FUNGI FROM DERMAL SITES

CREWMAN	SITE	TEST DAY															
		-4	4	11	18	25	32	34	39	46	53	60	67	74	81	88	+18
1	AX																
	PER		S														
	TW	P	A	A							A		A			A	H&A
2	AX																
	PER																
	TW			P								A	H		R		Y
3	AX													R	T		
	PER										R&T		R&Y		T		
	TW																
4	AX			D			A			A						A	
	PER																
	TW	D									A						T&Y

DERMAL SITES:

AX = AXILLA
PER = PERINEUM
TW = TOE WEB

ISOLATES:

A = ASPERGILLUS
D = Dematiaceous Mycelium
H = HORMODENDRUM
P = PENICILLIUM
R = RHODOTORULA
S = STACHYBOTRYS
T = TORULOPSIS
Y = Yeast, Unidentified

TABLE 7

IDENTIFICATION OF SABOURAUD DEXTROSE AGAR ISOLATES

STATION SURFACE SAMPLES

Isolate Code No.	Test Day	Area	Gross Morph. Type	Classification
H 116	-4	FM	14	Cephalosporium
H 118	-4	FM	22	Aspergillus
H 125	-4	H	22	Penicillium
H 128	18	H	24	Bacterium
H 179	39	H	25	Geotrichum (?)
H 177	46	H	26	Bacterium
H 200	53	H	25	Bacterium
H 203	53	FM	25	Bacterium
H 245	67	H	14	Pullularia
H 284	88	FM	22	Penicillium
H 285	88	H	14	Pullularia
DB 100	Post-test	H	19	Rhodotorula
DB 101	Post-test	H	6	Pullularia
DB 255	Post-test	FM	18	Pullularia

FM = Food Management Area

H = Hygiene Area

TABLE 8

RECOVERY OF FUNGI FROM STATION SURFACES

ISOLATE	SITE	TEST DAY														
		-4	4	11	18	25	32	39	46	53	60	67	74	81	88	POST-TEST
ASPERGILLUS	FM	X														
	H															
CEPHALOSPORIUM	FM	X														
	H															
GEOTRICHUM (?)	FM															
	H							X								
PENICILLIUM	FM														X	
	H	X														
PULLULARIA	FM															X
	H												X		X	X
RHODOTORULA	FM															
	H															X

AREA SAMPLED = 10 CM X 10 CM

FM = FOOD MANAGEMENT AREA

H = HYGIENE AREA

X = ORGANISM RECOVERED

FIGURE 1 - LOCATION OF POST-TEST SURFACE SAMPLES

Forward (Equipment) Compartment

Port	Starboard
Counter Behind Sabatier Reactor	*Vacuum Cleaner Hose, Interior
Thermal Control Duct Grill, Sample 1	Floor Under Psychomotor Tester, Right Pedal
Thermal Control Duct Grill, Sample 2	
Thermal Control Duct Air Return, Shelf	

Aft(Crew) Compartment →

Port	Starboard
Clothes Washer Drain Filter	Freezer Counter Top
Clothes Dryer Counter Top	Food Storage Area 74, Shelf
Microwave Oven Rear Counter Top	Floor Under Bunk
Spirometer Inlet	
Thermal Control Duct Grill, Sample 4	
Thermal Control Duct Grill 4, Top Corner	
Storage Area 1, Rear Corner	
Commode Seat, Exterior	
Urine Collector Debris Screen	
Volumetric Cylinder, Interior (Urine Measurement)	
Floor Under Urine Phase Separator	
Floor Under Bunk	

*Note: Vacuum Cleaner used throughout simulator, but stowed in forward compartment.

TABLE 9

IDENTIFICATION OF SABOURAUD DEXTROSE AGAR ISOLATES
POST-TEST SURFACE SAMPLES

Sampling Site	Isolate Code No.	Gross Morph. Type	Classification
Thermal Control Duct Grill - Sample 1	DB 75	26	Bacterium
	DB 76	21	Bacterium
	DB 136	22	Penicillium
	DB 137	25	Penicillium
Thermal Control Duct Grill - Sample 2	DB 138	26	Cryptococcus
	DB 139	22	Penicillium
Thermal Control Duct Grill - Sample 4	DB 258	18	dematiaceous mycelium
Thermal Control Duct Grill, 4, Top Corner	DB 65	25	Bacterium
	DB 125	20	Penicillium
Thermal Control Duct Air Return, Shelf	DB 130	13	Penicillium
	DB 131	20	Penicillium
Food Storage Area (74) Shelf	DB 119	22	Penicillium
Microwave Oven Rear Counter Top	DB 49	21	Bacterium
	DB 268	20	Penicillium
Freezer Counter Top	DB 58	25	Bacterium
	DB 116	16	Pullularia
Counter Behind Sabatier Reactor	DB 120	11	Penicillium
Clothes Dryer Counter Top	DB 55	25	Bacterium
	DB 56	25	Bacterium
	DB 113	19	Rhodotorula
	DB 114	7	Pullularia
Clothes Washer Drain Filter (Interior)	DB 134	19	Rhodotorula
Storage Area 1, Rear Corner	DB 64	11	Bacterium
	DB 123	20	Penicillium
Spirometer Inlet	DB 92	25	Bacterium
	DB 256	9	Aspergillus
Vacuum Cleaner Hose (Interior, Housing End)	DB 70	21	Bacterium
	DB 133	22	Penicillium
Urine Collector Debris Screen	DB 53	26	Torulopsis
	DB 54	25	Bacterium
	DB 110	19	Rhodotorula
	DB 111	26	Cryptococcus
Volumetric Cylinder, Urine Measurement (Interior Lip)	DB 57	25	Torulopsis
	DB 115	26	unidentified yeast
Commode Seat (Exterior)	DB 42	25	Bacterium
Floor under Urine Phase Separator	DB 67	25	Bacterium
	DB 127	23	Penicillium
	DB 128	24	Bacterium
	DB 129	14	Pullularia
Floor under Right Pedal of Psychomotor Tester	DB 132	14	Pullularia
Floor Under Port Bunk	DB 47	21	Bacterium
	DB 103	26	Cryptococcus
Floor Under Starboard Bunk	DB 48	21	Bacterium
	DB 106	20	Erodium

TABLE 10

FUNGI RECOVERED FROM SSS SURFACES POST-TEST

SITE	ISOLATE								
	ASPERGILLUS	CRYPTOCOCCUS	Dematiaceous Mycelium	ERODIUM	PENICILLIUM	PULLULARIA	RHODOTORULA	TORULOPSIS	Yeast Unidentified
THERMAL CONTROL DUCT GRILL - SAMPLE 1					X				
THERMAL CONTROL DUCT GRILL - SAMPLE 2		X			X				
THERMAL CONTROL DUCT GRILL - SAMPLE 4			X						
THERMAL CONTROL DUCT GRILL, 4, TOP CORNER					X				
THERMAL CONTROL DUCT AIR RETURN, SHELF					X				
FOOD STORAGE AREA (74) SHELF					X				
MICROWAVE OVEN REAR COUNTER TOP					X				
FREEZER COUNTER TOP						X			
COUNTER BEHIND SABATIER REACTOR					X				
CLOTHES DRYER COUNTER TOP						X	X		
CLOTHES WASHER DRAIN FILTER (INTERIOR)							X		
STORAGE AREA 1, REAR CORNER					X				
SPIROMETER INLET	X								
VACUUM CLEANER HOSE (INTERIOR, HOUSING END)					X				
URINE COLLECTOR DEBRIS SCREEN		X					X	X	
VOLUMETRIC CYLINDER, URINE MEASUREMENT (INTERIOR LIP)								X	X
FLOOR UNDER URINE PHASE SEPARATOR					X	X			
FLOOR UNDER RIGHT PEDAL, PSYCHOMOTOR TESTER						X			
FLOOR UNDER STARBOARD BUNK				X					
FLOOR UNDER PORT BUNK		X							

X = ORGANISM RECOVERED

AREA SAMPLED = 5 CM x 5 CM WHEREVER POSSIBLE

except for Cephalosporium and Geotrichum and the yeasts, Cryptococcus and Torulopsis. Erodium is the sexual stage of Aspergillus (imperfect or asexual stage) but is listed separately. Table 10 shows that Penicillium was the prevalent isolate for the thermal control duct system on both inlet and outlet sides. Penicillium and Pullularia and the yeasts, Cryptococcus, Rhodotorula and Torulopsis, were most frequently isolated from surface samples. Cryptococcus isolates were not C. neoformans but desert or environmental types.

Subsystem Components

Results for post-test samples of water subsystem components are presented in Table 11. Rhodotorula, Torulopsis and an unidentified yeast were the only fungal isolates. Schizosaccharomyces were the only fungal isolates from stored wastes (Table 12). Found in high sugar concentrations, they were not unusual isolates from the cans used to store jelly and condiment containers.

Air Samples

As shown in Tables 13 and 14, Aspergillus was the predominant fungal contaminant recovered from the atmosphere, with no difference between forward and aft sections observable. Penicillium was present throughout the run while Candida, 2 unidentified yeasts and Hormodendrum were isolated only during the latter part of the test (days 73-87). Even though the Candida isolate is an environmental type, it is not normally airborne. Table 15 (reproduced from Reference 1) shows the bacterial and fungal counts in the SSS atmosphere, which were low throughout the test. Counts for fungal types ranged from zero to 0.23 viable particles/1 ft^3. The counts may have been higher if the recovery medium and incubation temperatures were more conducive to fungal isolation (see Tables 1 and 2).

TABLE 11

IDENTIFICATION OF SABOURAUD DEXTROSE AGAR ISOLATES

POST-TEST SAMPLES OF THE POTABLE AND WASH WATER MULTIFILTRATION UNITS

Component	Isolate Code No.	Gross Morph. Type	Classification
Potable Water System			
Carbon 1 midstream	DB 262	25	Bacterium
Wash Water System			
Pall filter pore size 30 μm top	DB 230	26	unidentified yeast
	DB 231	25	Bacterium
Pall filter pore size 30 μm bottom	DB 222	26	Torulopsis
	DB 223	26	Bacterium
	DB 225	19	Rhodotorula
Pall filter pore size 3 μm bottom	DB 240	26	Bacterium
Pall filter pore size 1 μm bottom	DB 243	26	Bacterium
Mixed ion-resin midstream	DB 204	23	Bacterium
Carbon 1 midstream	DB 210	26	Bacterium
	DB 211	26	Bacterium
Carbon 2 outlet	DB 172	25	Bacterium

TABLE 12

IDENTIFICATION OF SABOURAUD DEXTROSE AGAR ISOLATES

POST-TEST SAMPLING OF STORED WASTE

Component		Isolate Code No.	Gross Morph. Type	Classification
Dry Waste (not quinolinol-treated)				
*Foil-wrapped bundle No.	4	DB 280	25	Bacterium
	5	DB 274	26	Bacterium
		DB 281	26	Bacterium
Wet Waste (quinolinol-treated)				
**Hermetically-sealed can No.	1	DB 275	26	Schizosaccharomyces
		DB 282	26	Schizosaccharomyces

*Freeze-dried food wrappers and cans, waste paper and gauze, test tubes.

**Can tops and bottoms, plastic tubing, wipes, jelly and condiment containers, table scraps.

TABLE 13

IDENTIFICATION OF SABOURAUD DEXTROSE AGAR ISOLATES

REYNIERS AIR SAMPLES

Isolate Code No.	Test Day	Section	Gross Morph. Type	Classification
DB 11	-4	F	21	Bacterium
DB 06	3	A	25	Bacterium
DB 07	17	A	22	Aspergillus
DB 08	17	A	22	Aspergillus
DB 10	17	F	21	Bacterium
DB 18	31	F	25	Bacterium
DB 19	31	F	22	Aspergillus
DB 20	31	A	20	Penicillium
DB 21	31	A	22	Aspergillus
DB 22	31	A	20	Penicillium
DB 26	44	F	22	Aspergillus
DB 23	45	A	20	Penicillium
DB 25	45	A	20	Penicillium
DB 27	45	F	20	Aspergillus
DB 29	45	A	20	Penicillium
DB 30	73	A	22	Aspergillus
DB 32	73	A	19	Rhodotorula
DB 33	73	A	19	Candida
DB 34	73	F	19	Rhodotorula
DB 35	73	F	25	unidentified yeast
DB 37	81	F	11	unidentified yeast
DB 77	87	A	13	Penicillium
DB 78	87	A	13	moniliaceous mycelium
DB 79	87	A	26	Penicillium
DB 80	87	F	15	Hormodendrum
DB 85	87	F	25	Bacterium
DB 86	87	A	19	Rhodotorula
DB 87	87	A	4	Bacterium

F = Forward (Equipment) Section
A = Aft (Crew) Section

TABLE 14

TYPES OF FUNGI RECOVERED FROM SSS ATMOSPHERE

ISOLATE	SITE	TEST DAY										
		-4	3	17	31	44	45	59	73	81	87	+4
ASPERGILLUS	F				X	X	X					
	A			X	X				X			
CANDIDA	F											
	A								X			
HORMODENDRUM	F										X	
	A											
Moniliaceous Mycelium	F											
	A										X	
PENICILLIUM	F											
	A				X		X				X	
RHODOTORULA	F								X			
	A								X		X	
Yeast, Unidentified	F								X	X		
	A											

NOTE: FORWARD AND AFT REYNIERS SAMPLERS WERE OPERATED SIMULTANEOUSLY AT 1 FT^3 OF AIR PER MIN. FOR 1 HR.

F = FORWARD (EQUIPMENT) COMPARTMENT

A = AFT (CREW) COMPARTMENT

TABLE 15

NUMBER OF VIABLE PARTICLES PER CUBIC FOOT IN THE SSS ATMOSPHERE

Test Day	Forward Equipment Section		Aft Crew Section	
	Bacterial	Fungal	Bacterial	Fungal
-1	0.07	0	0.02	0.02
3	0.70	0	0.16	0
17	1.92	0.05	1.05	0.03
31	1.00	0.03	1.22	0.02
45	0.73	0.12	2.07	0.23
59	1.83	0	1.67	0
73	3.00	0.08	0.83	0.07
80	2.87	0	2.13	0
87	2.57	0.03	0.90	0.05
+4	0.12	0	0.02	0

Note: Forward and aft Reyniers samplers were operated simultaneously at 1 ft^3 of air per min for 1 hr.

SECTION V

DISCUSSION

Possible changes in microflora indigenous to the microbiologically-closed SSS were surveyed during the 90-day Test. Fungi were of interest because of their potential implications in crew health and hardware biodeterioration. Since the majority of isolates were recovered by sampling and assay methods which emphasized isolation of medically-significant bacteria and clean room requirements were not imposed pretest, most fungi recovered were environmental in origin. They represent genera which are widely distributed in nature, rarely pathogenic, not fastidious in cultural requirements, hardy, and able to survive long periods of desiccation. No fungal buildup or die-off was observed; rather, data reflect the "survival" and wide dissemination during the 90-day test of types introduced into the SSS microecology pretest.

Crew samples yielded types indigenous both to humans and the environment, most prevalently the latter. No dermatophytes, such as Candida, were isolated. The recovery of Stachybotrys from Crewman #1 and the sudden (test mid-point) appearance of yeasts such as Rhodotorula and Torulopsis, were unusual and inexplicable findings. Coincidentally, Crewman #3 had shown a significant decrease in toe web anaerobic levels during the test mid-portion, reflecting treatment of his "athlete's foot" with Zephiran® and Tinactin® (Reference 1). No special effort was made to recover the causative agent of this infection.

Surface, subsystem component and atmospheric samples yielded mostly environmental types. Predominant isolates from surfaces constitute the 90-day test "background" list of fungal contaminants: Aspergillus, Penicillium, Pullularia, and Rhodotorula. However, the absence from this list of 1) phycomycetes like Mucor and Rhizopus, 2) Chaetomium, and 3) Streptomyces was particularly noteworthy. Even though bioassay methods for subsystem components were not particularly optimal

for post-test fungal recovery, it was apparent that gross fungal growth had not occurred (cf. Tables 11 & 12). Reference 1 presents a complete list of components tested. The small number of fungal types isolated from the water multifiltration units and stored wastes consisted of only yeasts: Rhodotorula, Torulopsis, Schizosaccharomyces and an unidentified type. More frequent sampling would have better defined the airborne picture, but no significant changes were observed in the cabin atmosphere. Buildup of certain airborne contaminants like Aspergillus and Penicillium could have resulted in the development of allergies or bronchitis. Finally, it was interesting to note that fungal contaminants in SSS environmental samples were predominantly environmental in origin. By contrast, bacterial contaminants in the same samples represented mainly human types (cf. Reference 1).

SECTION VI

CONCLUSIONS AND RECOMMENDATIONS

No fungal proliferation was noted that would jeopardize crew health and life support systems. The recovery spectrum for fungi was restricted to mainly hardy and non-fastidious environmental types, because cultural methods favored bacterial isolation, particularly in terms of media choice, incubation temperatures and moisturization. Dermal sampling and assay techniques were unsuitable for isolation of pathogenic types. Most fungal isolates in air, surface, and subsystem component samples were environmental types probably introduced into the SSS microecology pretest. Crew members were not major sources of fungi in the SSS environment as they were of bacterial contaminants.

For future testing, application of contamination control and cleanliness monitoring requirements during pretest operations would greatly reduce the number of environmental contaminants in the SSS microecology. Also during the pretest period, more thorough and extensive baseline data on background types could be obtained to better evaluate population shifts during the test. A fungal marker system would also be useful. Mycological assays of potable water samples would enhance monitoring of the water management system. Improved sampling and cultural techniques are required for a wider recovery spectrum from the crew and environment. Atmosphere monitoring should be more frequent, continuous, and correlated with crew/systems operations which generate microbial aerosols. Finally, to better evaluate potential materials/equipment deterioration, more quantitative and better-controlled studies on surface and subsystem contaminants are necessary.

SECTION VII

REFERENCES:

1. Operational 90-Day Manned Test of a Regenerative Life Support System. NASA CR-111881, May 1971.

2. Lodder, J. (Ed.). 1970. Chapter II. In: The Yeasts. A Taxonomic Study. Second edition. North-Holland Publishing Co., Amsterdam, The Netherlands.

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