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MICROBIOLOGICAL ANALYSIS OF DEBRIS FROM SPACE TRANSPORTATION SYSTEM (STS)-55 SPACELAB D-2

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13. ABSTRACT *(Maximum 200 words)*

Filter debris from the Spacelab module D–2 of STS–55 was analyzed for microbial contamination. Debris from cabin and avionics filters was collected by Kennedy Space Center personnel on May 8, 1993, 2 days postflight. Debris weights were similar to those of previous Spacelab missions. Approximately 5.1E+6 and 1.7E+5 colony forming units per gram of debris were enumerated from the cabin and avionics filter debris, respectively. These numbers were similar in previous missions for which the entire contents were analyzed without sorting of the material. Bacterial diversity was small compared to previous missions, with no gram negative bacteria isolated. Only one bacterial species, *Corynebacterium pseudodiphtheriticum*, was not isolated previously by the laboratory from Spacelab debris. This organism is a normal inhabitant of the pharynx. A table listing all species of bacteria isolated by the laboratory from previous Spacelab air filter debris collection is provided.

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INTRODUCTION

As part of an on-going study to determine the microbial content in the avionics and cabin environments of shuttle spacelab missions, air filter debris from STS-55, Spacelab-D2, was examined. Data gathered from these flights also provides information on the microbial diversity in air that may be expected during long-term missions.

In addition to reporting the results of the current analysis, a comparison with the 3 previous flights is presented. These flights included: STS-40,SLS-1; STS-42,IML-1; STS-49,SLJ. Debris weights, microbial counts and bacterial species identified from these flights are compared.

METHODS

Debris from the cabin and avionics filters was collected by Kennedy Space Center personnel on May 8, 1993, 2 days post-flight. Collection procedures have been described previously (1). Samples were delivered to the Microbial Ecology Facility housed in the Analytical and Physical Chemistry Branch of the Materials and Processes Laboratory at Marshall Space Flight Center approximately 8 days following shuttle landing.

The debris from the avionics and cabin filters was aseptically transferred to tared, sterile 500 ml polypropylene bottles and the debris weight recorded. A 100 ml volume of sterile phosphate buffered saline (PBS) was added to each bottle containing the debris and the contents sonicated for 15 minutes. After settling for 10 minutes, 1 ml of the cabin slurry was diluted as necessary in sterile PBS, and spread-plated on duplicate R2A plates. The avionics slurry was processed similarly. In an attempt to increase recovery of different bacterial species, aliquots of each sample were also inoculated initially in sterile 3% brain heart infusion broth (BHI) for 3 days at 28C. After this time, samples were diluted as appropriate, and spread-plated on R2A. All R2A plates were incubated at 28C for 7 days.

Identification of morphologically distinct bacterial colonies was determined by subculturing isolates onto trypticase soy agar (TSA) and/or Biolog Universal Growth Medium (BUGM) in preparation for identification using Minitek and Biolog microbial identification systems, respectively.

RESULTS

The total debris weight for the cabin and avionics debris was 5.84 grams and 3.46 grams, respectively. Table 1 is a comparison of debris weight between this flight and previous flights where total debris collected from the filters was analyzed by the laboratory. The debris weights for these 3 missions were similar.

Approximately 5 x 10^6 colony forming units/gram debris (cfu/g) were recovered from the cabin debris slurry plated directly onto R2A agar. The avionics sample slurry plated directly onto R2A contained 1.7 x 10^5 cfu/g. A comparison in microbial numbers to STS-49, SLJ, which was also examined using unsorted material, is shown in Table 2. There was no significant difference observed between the two missions.

Table 3 lists the bacterial species recovered from the cabin and avionics samples. The cabin debris sample plated directly on R2A agar consisted entirely of Staphylococcus aureus. The BHI-treated cabin sample contained no Staphylococcus but instead consisted of Bacillus sp. and Corynebacterium pseudodiphtheriticum. Four (4) species of Staphylococcus were recovered from the avionics debris sample plated directly on R2A agar. Bacillus sp. and Micrococcus luteus were also recovered from this sample. The only bacterial species recovered from the avionics debris sample incubated in BHI broth prior to plating on R2A agar was Staphylococcus aureus, which was also recovered by the direct plating method. All species had been recovered in the laboratory from previous spacelab missions except Corynebacterium pseudodiphtheriticum, a non-pathogenic bacterium normally isolated from the human pharynx.

Table 4 lists the bacterial species recovered by the laboratory from both the current and previous three spacelab missions. Greater bacterial diversity was found in STS 42, IML-1 and STS 49, SLJ, where the laboratory received all debris from the filters, compared to the partial collection in STS 40, SLS-1. Cabin filter debris from these three missions contained greater microbial diversity than the corresponding avionics filter debris.

In contrast, avionics filter debris from the current mission contained greater microbial diversity than the cabin filter debris. However, neither sample contained the diversity found in the IML-1 and SLJ missions, where total debris was also analyzed. The IML-1 and SLJ missions averaged 12 species in the cabin debris compared to 3 for the current mission. These previous missions also averaged 9 distinct species in avionics debris compared to 6 for this flight. This reduction in diversity in both cabin and avionics samples appears largely due to the absence of gram negative bacteria, which had been isolated on all previous missions by the laboratory.

DISCUSSION

Debris weights for both cabin and avionics samples were similar to those recorded in the 2 previous missions (IML-1 and SLJ), from which the laboratory received the entire debris collected from the filters. Post-flight analysis time was also similar for the 3 missions with analysis from this mission beginning 8 days following shuttle landing compared to 6 and 7 days for IML-1 and SLJ flights, respectively.

Total numbers of bacteria per gram of debris were also comparable to STS-49, SLJ, the only other mission from which the laboratory received the entire filter debris collected and analyzed the contents unsorted.

The use of BHI to improve recovery of bacterial species was inconclusive. This method appeared effective in recovery of distinct species from the avionics debris but not cabin debris. In the avionics debris sample, 6 distinct species were isolated in the BHI-inoculated sample. Only 1 species was isolated from direct plating onto R2A agar, and this species was also found in the BHI-inoculated sample. A total of three distinct species were isolated from cabin samples, two from the BHI broth samples and one from direct plating onto R2A agar.

Overall bacterial diversity was small in both cabin and avionics debris samples in comparison to previous missions, largely due to the absence of gram negative bacteria. Because debris weights and processing times were similar for all 3 missions where the total debris was analyzed, it is unlikely that the absence of gram negative bacteria represents an actual decrease in these bacteria in both cabin and avionics air systems during the flight. It is more likely that environmental changes such as excess heat and drying during transport to the laboratory led to the loss of the gram negative population.

Only one bacterial species, Corynebacterium pseudodiphtheriticum, has not been isolated from previous spacelab missions by the laboratory. This organism is a normal inhabitant of the pharynx.

REFERENCES

1. Summary of Reports Detailing Analysis of Debris Collected From SLS-1 Fan Filters in June and July, 1991. E.B. Rodgers and M.C. Roman, NASA/MSFC, Huntsville, AL 35812.

TABLE 1

Comparison of Avionics and Cabin Debris
Weights Between Spacelab Flights

MISSION	DEBRIS WEIGHT (GRAMS)	
	CABIN	AVIONICS
STS-42, IML-1	8.51	2.3
STS-49, SLJ	3.61	4.69
STS-55, D-2	5.84	3.46

TABLE 2

Comparison in Microbial Numbers Between
STS-49, SLJ and STS-55, D-2

MISSION	COLONY FORMING UNITS/GRAM DEBRIS	
	CABIN	AVIONICS
STS-49, SLJ	7.7E+6	7.6E+4
STS-55, D-2	5.1E+6	1.7E+5

TABLE 3

Bacterial Species Recovered From Cabin
and Avionics Debris Using R2A and BHI Broth

CABIN	**AVIONICS**
R2A	R2A
Staphylococcus aureus	*Staphylococcus aureus* *Staphylococcus sp.* *Staphylococcus epidermidis* *Staphylococcus cohnii* *Micrococcus luteus* *Bacillus sp.*
BHI Broth	BHI Broth
Bacillus sp. *Corynebacterium pseudodiphtheriticum*	*Staphylococcus aureus*

TABLE 4 MICROORGANISMS RECOVERED FROM SPACELAB AIR FILTER DEBRIS

MICROORGANISMS	MISSION							
	STS-40 (a)		STS-42 (b)		STS-49 (c)		STS-55 (c)	
	CABIN	AVIONICS	CABIN	AVIONICS	CABIN	AVIONICS	CABIN	AVIONICS
Acinetobacter lwoffi				√				
Actinobacillus seminis					√			
Bacillus insolitus					√	√		
Bacillus sp.	√	√	√	√			√	√
Brucella abortus biovar 2					√			
Citrobacter freundii			√					
Citrobactyer amalonaticus			√					
Clavebacterium michingaensis					√			
Corynebacterium minutissimum						√		
Corynebacterium pseudodiphtheriticum							√	
Enterobacter agglomerans	√		√	√				
Enterobacter asburiae					√			
Enterococcus faecalis					√			
Escherichia hermanii					√			
Haemophilus aphrophilus						√		
Klebsiella ozaenae			√	√				
Klebsiella pneumoniae A					√	√		
Klebsiella terrigena					√			
Methylobacter sp.	√							
Methylobacterium extorquens						√		
Micrococcus luteus	√							√
Micrococcus naucinus					√			
Moraxella sp.	√							
Pseudomonas aeruginosa				√				
Pseudomonas fluor/Putida				√				
Pseudomonas fluorescens B					√	√		
Pseudomonas paucimobilis		√						
Pseudomonas pickettii			√					
Pseudomonas vesicularis			√					
Staphylococcus aureus			√	√	√	√	√	√
Staphylococcus cohnii								√
Staphylococcus epidermidis			√	√		√		√
Staphylococcus haemolyticus	√		√	√				
Staphylococcus hominis					√			
Staphylococeus sp.								√
Streptococcus faecalis			√					
Streptococcus faecium D			√					
Strephtococcus sp.								√
Xanthomonas campestris						√		

a= partial debris collection, material sorted
b= total debris collection, material sorted
c= total debris collection, material unsorted

APPROVAL

MICROBIOLOGICAL ANALYSIS OF DEBRIS FROM SPACE TRANSPORTATION SYSTEM (STS)–55 SPACELAB D–2

By Timothy L. Huff

The information in this report has been reviewed for technical content. Review of any information concerning Department of Defense or nuclear energy activities or programs has been made by the MSFC Security Classification Officer. This report, in its entirety, has been determined to be unclassified.

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