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Microbiological Profiles of the Viking Spacecraft

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Planetary quarantine requirements associated with the launch of two Viking spacecraft necessitated microbiological assessment during assembly and testing at Cape Canaveral and the Kennedy Space Center. Samples were collected from selected surfaces of the Viking Lander Capsules (VLC), Orbiters (VO), and Shrouds at predetermined intervals during assembly and testing. Approximately 7,000 samples were assayed. Levels of bacterial spores per square meter on the VLC-1 and VLC-2 were 1.6×10^2 and 9.7×10^1, respectively, prior to dry-heat sterilization. The ranges of aerobic mesophilic microorganisms detected on the VO-1 and VO-2 at various sampling events were 4.2×10^2 to 4.3×10^3 and 2.3×10^2 to $8.9 \times 10^3/m^2$, respectively. Approximately 1,300 colonies were picked from culture plates, identified, lyophilized, and stored for future reference. About 75% of all isolates were microorganisms considered indigenous to humans; the remaining isolates were associated with soil and dust in the environment. The percentage of microorganisms of human origin was consistent with results obtained with previous automated spacecraft but slightly lower than those observed for manned (Apollo) spacecraft.

On August 20 and September 9, 1975, two Viking spacecraft were launched from Cape Canaveral, Fla. The mission of these unmanned spacecraft is the scientific exploration of the planet Mars, with special emphasis on the search for extraterrestrial life. A miniature, automated biological laboratory, The Viking Lander Biology Instrument, will be used to detect life on the Martian surface (11). The first of the Viking spacecraft is scheduled to land on Mars in July 1976.

To insure that no terrestrial microorganisms would be transported to the planet via the spacecraft, both spacecraft were assembled and tested under controlled environmental conditions to reduce the microbial contamination on the spacecraft surfaces. This was in compliance with an international agreement that required that the probability of contaminating the planet Mars be less than $^1/_{1,000}$ during the period of biological exploration (9, 10, 15), a requirement that led to a United States allocation of 2×10^{-4} for both Viking spacecraft (17). In addition, each Viking Lander Capsule (VLC) was subjected to a terminal dry-heat sterilization cycle. The cycle consisted of a nominal temperature of 111.7 ± 1.7°C for a period of 23 to 30 h after the coldest contaminated point reached 111.7°C in an inert environment of nitrogen gas having an oxygen content of less than 2.5% and a moisture content defined as less than 0.097% by weight (28). The duration of each cycle was dictated by the level of aerobic mesophilic bacterial spores present on the VLC prior to sterilization. The above measures successfully assured that the allocated probabilities of contaminating Mars were met.

The objective of this study was to perform a prelaunch microbiological assay of the Viking spacecraft for the purpose of quantitatively estimating or verifying microbial contamination reduction.

MATERIALS AND METHODS

Assays were conducted on the Viking spacecraft during assembly and testing at Kennedy Space Center and Cape Canaveral, Fla., from January to August 1975. Sampling sites were selected on the interior and exterior surfaces of the various spacecraft components, which consisted of the VLC, Viking Orbiter (VO), and Shroud by criteria defined in the Viking '75 Program Microbiological Assay and Monitoring Plan (16). The spacecraft components were assayed at predetermined intervals during assembly and testing. At each interval, 250 locations on each spacecraft component were sampled and assayed by methods previously described by Puleo et al. (23-26). Samples taken from the spacecraft were assayed by the respective organization and the Planetary Quarantine Laboratory (PQL). The PQL was responsible for assaying 50% of all samples taken as described in the Viking '75 Plan (15).

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All assays were performed in three separate laboratory areas located in PQL. These laboratories housed the bioassay teams from Martin-Marietta Aerospace, Jet Propulsion Laboratory Viking Orbiter, and PQL. The bioassay teams consisted of two people, a microbiologist from the responsible organization and a microbiologist provided by PQL. PQL had two bioassay teams; one team assayed only VO samples and the other team assayed the VLC samples.

Sterile cotton swabs, moistened in 10 ml of sterile distilled water, were rubbed over the surfaces to be sampled, which were outlined with a sterile aluminum template (12.9 cm^2). Surface areas smaller than 12.9 cm^2 were determined by direct measurement. The swab was returned to the original screw-cap test tube (20 by 150 mm) containing 10 ml of sterile distilled water. The swab head was broken off below the portion of the handle touched by the sampler. Tubes were taken to the laboratory, agitated on a Vortex mixer for 5 to 6 s, placed in an ultrasonic bath (tank LTH60-3; generator, A-300; Branson Instruments, Inc., Stamford, Conn.) containing a 0.3% (vol/vol) solution of Tween 80 (polyoxethylene sorbitan monooleate; Hilltop Research, Inc., Miamiville, Ohio), and insonated for 2 min at 25 kHz (13, 21, 22). After insonation, 4 ml of rinse solution from each tube was plated with Trypticase soy agar (BBL, Cockeysville, Md.). The remaining liquid in each tube was placed in a water bath and heat shocked at 80 ± 2°C for 20 min. After heat shock, 4 ml of the remaining sample was plated with Trypticase soy agar. All samples were assayed within 1 h after being taken. Culture plates were incubated at 32°C under aerobic conditions for 3 days. Because of the low colony counts obtained, a stereozoom microscope was used to verify and confirm each colony count.

In addition to the 250 samples, 25 sterile controls (10%) were included for each sampling period. These controls challenged both the sampling and assay procedures. Sterility checks were done on 5% of all supplies and materials used in the assay procedure prior to their use. Bacteriological media were incubated for a minimum of 3 days prior to use. Semiannual calibration or certification (or both) was required of laboratory equipment, such as incubators, water baths, thermometers, and laminar-flow clean benches.

All laboratory procedures were performed in a horizontal laminar-flow clean bench (6) to eliminate background airborne contamination. Other precautions taken to insure against extraneous contamination included: the use of sterile gloves by bioassay personnel while processing samples; use of media control plates; sterilization of all liquids that would come into contact with the test tube containing the sample; and rigorous control of personnel density in the PQL during assays.

Micrococcaceae were classified by the scheme of Baird-Parker (2), and aerobic sporeformers (*Bacillus* spp.) were classified by a modified version of the method of Gordon et al. (8). *Lactobacillaceae* were classified by the schemes of Sharpe et al. (27). *Bergey's Manual of Determinative Bacteriology* (7th ed.) was used for classifying other groups of bacteria.

RESULTS AND DISCUSSION

Bioassays were performed on the Viking Precursor, Viking 1, and Viking 2 spacecraft. The Precursor spacecraft was a flight orbiter and lander used to verify spacecraft level flight article assembly and test operating procedures at the launch site. Viking 1 spacecraft consisted of VLC-1, VO-1, and Shroud B. Viking 2 spacecraft consisted of VLC-2, VO-2, and Shroud A.

Table 1 shows the number of swab samples taken during the assembly of the various Viking spacecraft. A total of 6,683 samples were taken and assayed. Of these, 624 were negative control samples. These included both handling (sterile water blanks containing sterile cotton swab heads) and sampling (sterile cotton swab moistened in 10 ml of sterile distilled water and then broken off below the portion of the handle touched by the sampler) controls. Controls were processed in the laboratory in the same manner as spacecraft hardware samples. These negative controls constituted a minimum of 10% of the total samples taken at each sampling operation.

Data from the VO assays are presented in Table 2. The levels of aerobic mesophilic microorganisms and spores per square meter of surface sampled were found to be relatively low. Because the front surfaces of the solar panels from each VO were sampled once, the results obtained were included in all calculations to determine the number of microorganisms per square meter obtained at the various sampling periods. Table 3 shows the results of microbial assays on the VLC and Shrouds. The levels of

TABLE 1. *Number of samples assayed for the Viking missions*

Source	No. of samples taken	No. of controls	Total no. assayed
Precursor			
VLC-1	990	104	1,094
VO-1	325	33	358
Shroud B	250	25	275
Solar panels	52	5	57
Subtotal	1,617	167	1,784
Viking 1			
VLC-1	1,003	103	1,106
VO-1	594	60	654
Shroud B	250	25	275
Subtotal	1,847	188	2,035
Viking 2			
VLC-2	1,251	134	1,385
VO-2	792	80	874
Shroud A	500	50	550
Solar panels	52	5	57
Subtotal	2,595	269	2,864
Total	6,059	624	6,683

aerobic spores detected on the VLC prior to terminal sterilization were greater than those detected on the VO. The Precursor was found to contain the highest number of spores per square meter. Prior to terminal sterilization, samples were assayed only for mesophilic aerobic bacterial spores. Subsequent to sterilization, samples taken from the bioshield exterior surfaces were assayed for mesophilic aerobic vegetative microorganisms and spores. The time interval for terminal sterilization was determined from the results obtained from the second sampling event (Table 3).

Bacterial colonies from each assay were randomly selected and picked for identification. A total of 294, 460, and 540 colonies was isolated from the Precursor, Viking 1, and Viking 2, respectively. These included organisms recovered from the non-heat-shocked portion of the sample as well as those organisms that survived heat shocking. One thousand, two hundred and ninety-four colonies were picked throughout the program for identification; of these, 951 were vegatative microorganisms and 343 were *Bacillus* spp. After identification, these microbial isolates were lyophilized and stored at −20°C for future reference.

Each organism was examined microscopically and placed into one of several large groups. Further testing along with computer analysis (3) of the data collected assigned each organism to a more specific group. The percentages and types of organisms identified from each spacecraft are shown in Table 4.

TABLE 2. *Microbial contamination detected on VO*

Source	Sampling events	No. of samples	No. of microorganisms/m²	
			Aerobes[a]	Aerobic spores[b]
VO-1 (P)[c]	1	185	2.5×10^3	8.6×10^1
	2	140	8.6×10^2	6.5×10^1
Solar panels		52	4.8×10^2	5.4×10^1
VO-1	1	250	4.3×10^3	4.3×10^1
	2	250	1.6×10^3	2.2×10^1
	3	250	4.2×10^2	4.3×10^1
Solar panels		52	2.0×10^2	9.7×10^1
VO-2	1	250	8.9×10^3	1.4×10^2
	2	250	1.2×10^3	3.2×10^1
	3	250	2.3×10^2	4.3×10^1
	3[d]	250	5.1×10^2	2.2×10^1

[a] Samples were not heat shocked; aerobic incubation. The count includes solar panel (front surface) counts.
[b] Samples were heat shocked; aerobic incubation. The count includes solar panel (front surface) counts.
[c] P, Precursor.
[d] Recycle. Samples were retaken because of spacecraft problems.

TABLE 3. *Microbial contamination detected on VLC and Shrouds*

Source	Sampling events	No. of samples	No. of microorganisms/m²	
			Aerobes[a]	Aerobic spores[b]
VLC-1 (P)[c]	1	250	–	2.4×10^2
	2	251	–	8.0×10^2
	3	293	1.1×10^3	2.0×10^2
	4	196	2.0×10^4	1.4×10^2
Shroud B (P)	1	250	6.8×10^2	2.2×10^1
VLC-1	1	253	–	1.5×10^2
	2	250	–	1.6×10^2
	3	250	2.3×10^2	3.2×10^1
	4	250	1.3×10^2	5.4×10^1
Shroud B	1	250	2.4×10^2	4.3×10^1
VLC-2	1	250	–	2.3×10^2
	2	250	–	9.7×10^1
	3	250	2.4×10^3	4.3×10^1
	4	250	2.4×10^2	2.2×10^1
Shroud A	1	250	1.3×10^2	5.4×10^1
VLC-2	4R[d]	251	1.8×10^2	1.1×10^1
Shroud A	1R[d]	250	2.0×10^3	3.2×10^1

[a] Samples were not heat shocked; aerobic incubation.
[b] Samples were heat shocked; aerobic incubation.
[c] P, Precursor.
[d] Recycle. Samples were retaken because of spacecraft problems.

Although psychrophilic microorganisms have been isolated from soil samples obtained from assembly areas associated with the Viking spacecraft at Cape Canaveral (7), none were detected on the spacecraft surfaces. Efforts by T. L. Foster of Hardin-Simmons University to isolate psychrophilic and mesophilic obligate anaerobes from the heat-shocked portions of the samples were also unsuccessful (T. L. Foster, personal communication.)

Members of the genus *Bacillus* were the most frequently isolated organisms during assay of the Precursor (Table 4). These accounted for slightly more than 47% of the total isolates identified, a figure that represents more than twice the number of any other group isolated.

More than 55% of the organisms detected on the Viking 1 spacecraft (Table 4) were gram-positive cocci (*Staphylococcus* spp. and *Micrococcus* spp.). These organisms are characteristic of those being indigenous to human skin, hair, and respiratory tract and are constantly being expelled from the human body. The levels of spore-formers (*Bacillus* spp.) and *Actinomycetes*, which are associated with soil and

TABLE 4. *Percentages and types of microorganisms detected on the Viking spacecraft*[a]

Type	VLC			VO			Shroud		
	Pre-cursor	VLC-1	VLC-2	Pre-cursor	VO-1	VO-2	Pre-cursor	Viking 1	Viking 2
Staphylococcus spp.									
Subgroup II	1	2	8	4	10	3	0	10	2
Subgroup III	0	0	1	0	2	<1	0	0	0
Subgroup IV	0	7	3	4	8	23	4	5	10
Subgroup V	1	0	2	0	5	7	0	10	20
Subgroup VI	0	0	0	0	1	1	0	0	0
Micrococcus spp.									
Subgroup 1	2	2	2	0	4	2	13	0	9
Subgroup 2	0	4	0	0	1	<1	4	0	3
Subgroup 7	9	18	27	4	17	10	0	10	4
Subgroup 8	0	5	0	0	0	0	0	0	1
Atypical *Micrococcus*	6	7	8	9	11	23	25	5	8
Streptococcus-Enterococcus group	0	0	0	0	0	<1	0	0	0
Bacillus spp.									
B. alvei	0	4	1	0	<1	0	0	5	0
B. brevis	4	0	1	0	1	1	0	0	0
B. cereus	1	4	2	0	1	1	0	0	1
B. circulans	0	0	2	4	1	<1	4	5	0
B. coagulans	0	0	1	0	0	0	0	0	0
B. firmus	1	2	1	0	2	<1	4	0	1
B. lentus	8	9	5	13	5	4	8	10	9
B. licheniformis	1	0	1	0	0	0	0	0	0
B. macerans	0	0	1	0	0	0	0	0	0
B. megaterium	1	0	0	2	2	1	4	0	0
B. polymyxa	0	0	0	0	0	<1	0	0	0
B. pumilus	6	4	1	0	<1	<1	4	0	3
B. sphaericus	2	0	0	2	0	<1	0	0	0
B. subtilis	14	5	4	7	1	1	13	10	7
Atypical *Bacillus*	11	5	7	13	7	4	8	20	18
Corynebacterium-Brevibacterium group	21	16	16	33	17	13	4	10	3
Actinomycetes	4	2	7	4	1	1	4	0	0
Yeasts	7	4	0	0	3	<1	0	0	1
Total no. isolated	143	55	115	45	252	206	26	18	91

[a] Includes only non-heat-shocked, aerobic, mesophilic microorganisms.

dust, were found to be low when compared with levels for other automated spacecraft.

The profile of organisms detected on the Viking 2 spacecraft (Table 4) was very similar to that seen on Viking 1. *Micrococcus* spp. and *Staphylococcus* spp. constituted the greater percentage of microorganisms isolated from VO-2. A previously undetected microorganism belonging to the genus *Streptococcus*, and probably of human origin, was isolated from the Orbiter. A greater diversity of species was seen among the *Bacillus* identified.

The above data do not reflect the members of the genus *Bacillus* that were isolated and identified from heat-shocked assay samples. Although these data were not reflected in the microbial profiles of each spacecraft sampled (Table 4), they were collected and compared to the *Bacillus* obtained from non-heat-shocked samples. The only major difference was in the frequency of isolation of organisms identified as *Bacillus subtilis*. Approximately 17% of the *Bacillus* spp. isolated and identified from non-heat-shocked portions of assay samples was classified as *B. subtilis*. The number of organisms identified as *B. subtilis* from heat-shocked portions of the samples dropped noticeably and accounted for only 8% of the *Bacillus* spp. identified. More than 50% of the organisms recovered, whether from heat-shocked or non-heat-shocked portions of assay samples, were distributed into two major groups, namely, *Bacillus lentus* and the atypical *Bacillus* spp. This distribution is not uncommon and was observed in previous assays of spacecraft.

A comparison of the percentage and types of microorganisms isolated from the Viking spacecraft is shown in Table 5. Approximately 75% of

TABLE 5. *Comparison of the percentages[a] and types of microorganisms isolated from the Viking spacecraft*

Type of microorganism	Microorganisms on spacecraft (%)		
	Precursor	Viking 1	Viking 2
Micrococcaceae	22.9	55.4	61.3
Bacillus spp.	47.2	23.9	22.9
Corynebacterium-Brevibacterium group	21.4	16.8	12.0
Actinomycetes	4.2	0.9	2.8
Yeasts	4.7	2.7	0.5
Enterococcus	0	0	0.2

[a] Includes only non-heat-shocked, aerobic, mesophilic microorganisms.

isolates detected on Viking 1 and 2 spacecraft were microorganisms that are considered to be indigenous to humans (*Micrococcus* spp., *Staphylococcus* spp., and *Corynebacterium-Brevibacterium* group). The remaining microorganisms were associated with soil and dust in the environment. The Precursor, however, was found to have a greater percentage of those microorganisms associated with soil and dust.

It is evident from the results obtained that the levels and types of microorganisms on surfaces of the Viking 1 and 2 spacecraft remained relatively constant and were similar to those found on prior automated spacecraft (5, 20; N. D. Fields, J. R. Puleo, B. Moore, and R. C. Graves, Proc. Annu. Meet. Am. Assoc. Contam. Contr., 7th, Chicago, Ill., 1968) but low in comparison with those found on Apollo spacecraft (23–26). The Precursor spacecraft was found to have the highest levels of microbial contamination, and a greater percentage of those mcroorganisms was associated with soil and dust. The assembly of the spacecraft in a rigidly controlled, class 100,000 environment and stringent clean room procedures probably accounted for the low levels of microbial contamination observed. No significant differences were observed in the types of microorganisms found on Viking 1 and 2. The distribution patterns were the same when one compared the same component hardware (i.e., VLC-1 to VLC-2, VO-1 to VO-2, etc.).

The total numbers of aerobic mesophilic bacterial spores on VLC-1 and VLC-2 during the sampling period prior to lander encapsulation and terminal sterilization were calculated to be 2.54×10^5 and 2.01×10^5, respectively (17). These figures include the spore load of the insulation material. To obtain the final bioload on the spacecraft, the quantitative results were adjusted by a factor of two to compensate for the 50% efficiency factor of the swab-rinse technique (12). This approach was taken because the removal and recovery efficiency factor of the swab-rinse technique has been reported by some investigators to be approximately 50% (1, 4). When estimating the levels of microbial contamination on the VO and VLC surfaces shown in Tables 2 and 3, the 50% efficiency factor was not considered in the mathematical computation used to obtain those quantitative results. However, the efficiency factor was used to establish the microbial levels that determined the time at temperature for the terminal sterilization cycle.

In summary, the data show that low levels of microbial contamination were maintained on the Viking spacecraft prior to and after terminal heat treatment. The types of microorganisms detected were similar to those found on previous automated spacecraft.

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