20p.

N64 11293

CODE-1

NASA CR 52841

IIT RESEARCH INSTITUTE

formerly Armour Research Foundation of Illinois Institute of Technology

[redacted]

Technology Center Chicago, Illinois 60616

OTS PRICE

XEROX $ _____

MICROFILM $ _____

Report No. IITRI 3194-11
(Quarterly Status Report)

LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

National Aeronautics
and Space Administration
Washington, D.C.

UNPUBLISHED PRELIMINARY DATA

IIT RESEARCH INSTITUTE

auth [1463]
p.18

(NASA CR- -- ; Report No. IITRI-C194-11) OTS: (cover)
(Quarterly Status Report)

LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

September 15 to November 15, 1963

National Aeronautics and Space Administration

(NASA Contract No. NASr-22;
IITRI Project C194)

I. INTRODUCTION

Isolation of bacteria from soils exposed to severe environments have continued. Arrangements have been made with Dr. Emanuel Rudolph, Institute of Polar Studies, Ohio State University to provide lichen and soil samples from Antarctica and with Dr. William Boyd, Department of Microbiology, Ohio State University to provide pure cultures of bacteria and soil samples obtained from his recent field trip to the Arctic. The indigenous bacterial populations in soils from Rocky Mountain National Park (Colorado) and the White Sands National Monument (New Mexico) have been counted. Facultative anaerobic bacteria isolated from these soils in addition to facultative anaerobes previously isolated from California desert soils are being studied for their ability to survive the simulated Martian environment.

Twenty-nine facultative anaerobes isolated from the California desert soils, have been screened for survival in the simulated Martian environment. Results on the survival of 17 of these isolates are presented in this report. With few exceptions the organisms have survived the initial 28-day exposure period. The majority of these bacteria are Gram-positive, sporulating bacilli.

One of these isolates, an organism from California desert soil No. 68, identified as Bacillus cereus var. mycoides, is being studied for survival in the simulated Martian environment for prolonged exposure periods. Anaerobic and aerobic plate counts were made to determine any change in the microorganism's oxygen requirement resulting from exposure to the simulated Martian environment. At this time no significant difference has been noted. The anaerobic counts are slightly higher than the aerobic counts, but both are decreasing in a similar manner as the exposure period is extended. However, 64 and 70% of the cells, recovered as total and spore counts, respectively, have survived 56 days.

Survival of B. subtilis var. globigii as a function of moisture is presently being examined. B. subtilis inoculated into Martian soil, containing 1% organic medium, and varying concentrations of moisture have been exposed to the simulated Martian environment. After 14 days, the percent survival of both vegetative and spore cells was higher in the soils containing the higher concentrations of moisture.

Experiments to isolate the fungal component from lichens have been initiated. Apothecia germination, mycelial growth, and pigment production of the fungal component, while exposed to the simulated Martian environment are being studied.

II. EXPERIMENTAL PROCEDURES

The simulated Martian atmosphere described in Report 3194-5 was used. The methods of growing, harvesting, inoculating, and sampling the Mars tubes and the types of media used were described in Reports 3194-2, -3, -4, -7, and -10.

The method for obtaining various concentrations of moisture in the Mars tubes was by diluting the culture used for inoculum. The standard inoculum is 10^6 to 10^8 cells/0.01 ml. By appropriate dilution the same number of bacteria was contained in 0.05, 0.1 and 0.5 ml of water.

III. RESULTS AND DISCUSSION

Tables 1 and 2 give bacterial counts on trypticase soy agar of soil samples collected from tundra, and desert environments, respectively. Generally, the numbers of bacteria were higher in the tundra than desert soil samples. All samples were collected from the top 12 inches which usually contain the greatest numbers of bacteria. The anaerobic bacteria from both tundra and desert soils were more frequently sporulating bacilli. A greater number of cocci was isolated from the desert than tundra soils whereas a greater number and variety of pigmented

Table 1

BACTERIAL COUNTS OF TUNDRA SOILS*

Sample No.	Location**	Number of Bacteria/g			
		Aerobic		Anaerobic	
		Total Count	Spore Count	Total Count	Spore Count
1	Iceberg lake area alt. 12,200 ft volcanic soil	106×10^2	20×10^2	13×10^2	7×10^2
2	Similar to sample No. 1	14×10^4	22×10^3	15×10^3	12×10^3
3	Trailridge road area alt. 12,000 ft.	152×10^4	66×10^2	71×10^2	50×10^2
4	Niwot Mt. Colorado alt. 12,000 ft Uni. of Colorado Alpine and Artic Research Institute	23×10^5	49×10^4	45×10^4	90×10^2

*Soils collected at Rocky Mountain National Park, Colorado.
**All samples were surface soils with the top 1 inch of soil removed.

Table 2

BACTERIAL COUNTS OF DESERT SOILS*

Sample No.	Location**	Number of Bacteria/g			
		Aerobic		Anaerobic	
		Total Count	Spore Count	Total Count	Spore Count
1	Nonvegetative area 1 in. below surface	27×10^4	63×10	2×10	2×10
2	Same as No. 1, 10 in. below surface	41×10^4	112×10^2	4×10	6×10
3	Vegetative area 1 in. below surface	145×10^3	81×10	92×10	38×10
4	Same as No. 3, 12 in. below surface	23×10^3	27×10^2	28×10	16×10

*Soils collected at White Sands National Monument, New Mexico.
**All samples were collected from position 8, 1 mile west of road.

bacterial types were isolated from the tundra soils. The predominating bacterial type, isolated from both tundra and desert soils, were Gram-positive, sporulating bacilli. Since the soil of the White Sands National Monument is approximately 99% calcium sulfate several concentrations of sterile calcium sulfate were added to trypticase soy agar. The addition of calcium sulfate had no effect on maximal recovery of bacteria from these soil samples.

Table 3 shows the number of facultative anaerobic bacteria isolated from three different regions: California and New Mexico deserts and Colorado tundra region. Several criteria were used to facilitate studies with the large numbers of isolates from these soils. Initially, colonial morphology on trypticase soy agar was described and a Gram stain was made. The isolates were then grouped according to their oxygen requirement and the facultative anaerobic organisms were tested for lecithinase production. Gram-positive bacilli were the predominating bacteria isolated from all soils.

Results from experiments with 17 cultures, isolated from California desert soils, for survival in the simulated Martian environment for 28 days are presented in Table 4. With two exceptions, cultures 21 and 63, all cultures survived.

Table 3

ISOLATES OBTAINED FROM SOIL SAMPLES

Source	Number of Soil Samples	Number of Isolates	Number of Facultative Anaerobes	Number of Lecithinase Pos.
California	5	253	88	79
Colorado	4	17	15	11
New Mexico	4	40	32	17

Table 4

SURVIVAL AFTER 28 DAYS IN THE MARTIAN ENVIRONMENT OF MICROORGANISMS ISOLATED FROM CALIFORNIA DESERT SOILS

Culture No.	Source**	Organism	Number of Bacteria/g* 0 Day Total Count	0 Day Spore Count	28 Days Total Count	28 Days Spore Count
1	51	Bacillus	108×10^3	50×10^4	30×10^4	15×10^4
13	51	Bacillus	22×10^4	22×10^4	54×10^4	20×10^4
14	51	Bacillus	22×10^5	14×10^5	33×10^5	38×10^4
21	62	Bacillus	10^3	10^3	10^3	10^3
40	68	Bacillus	80×10^4	50×10^4	106×10^4	36×10^4
47	68	Bacillus	68×10^5	35×10^5	56×10^5	19×10^5
51	68	Coccus	141×10^5		106×10^5	
63	70	Bacillus	43×10^3	10^3	10^3	10^3
69	70	Bacillus	46×10^5	21×10^4	33×10^4	25×10^4
70	70	Bacillus	24×10^5	37×10^4	43×10^4	29×10^4
75	70	Bacillus	69×10^5	37×10^5	77×10^5	30×10^5
100	76	Bacillus	35×10^4	18×10^4	36×10^4	30×10^4
101	76	Bacillus	93×10^5	78×10^5	108×10^5	118×10^5
102	76	Bacillus	78×10^5	47×10^5	62×10^5	53×10^5
105	76	Bacillus	86×10^5	28×10^4	77×10^5	27×10^5
107	76	Bacillus	32×10^3	60×10^3	19×10^3	59×10^3
108	76	Bacillus	95×10^4	29×10^4	77×10^4	36×10^4

*Desert soil No. 62 was used as the substrate in the screening experiments.

**Desert soils supplied by Dr. R. E. Cameron of Jet Propulsion Laboratories, California Institute of Technology.

50 - Sand
62 - Sandy Loam
68 - Clay
70 - Sand
76 - Sand

Survival in the simulated Martian environment of an organism isolated from A california desert soil (No. 68) is illustrated in Figure 1. The organism has been identified as B. cereus var mycoides. After a 56-day exposure, 56 and 83% of the cells from the Earth control group were recovered as total and spore counts respectively, while 64 and 70% of the cells from the Martian experimental group were recovered as total and spore counts respectively. Except for a flucuation in numbers of bacteria after 7 days exposure there was no significant difference in survivability between the control and experimental groups. B. subtilis var globigii is presently being studied for survival in a simulated Martian environment modified by the incorporation of 1% organic medium and varying concentrations of moisture. Figure 2 illustrates the effect on survival by increasing the soil moisture from 1% to 2%. Bacterial counts are shown in Table 5. The 2% moisture does not appear to have influenced the survival of the bacteria. There is an indication, but not sufficient data, that the increase in total count for the Earth Control represents growth.

Figure 3 and Table 6 demonstrates the effect of higher moisture levels on the survival of B. subtilis. It has been established that moisture concentrations of 4.90 and 21.7% had an effect on the survival of this microorganism. Essentially greater numbers of bacteria survived the flushing and inoculating procedures in the tubes containing the higher soil moistures. There appears to be a relationship between the soil moisture

Fig. 1

Survival of Bacillus Cereus var. Mycoides in the Simulated Martian Environment

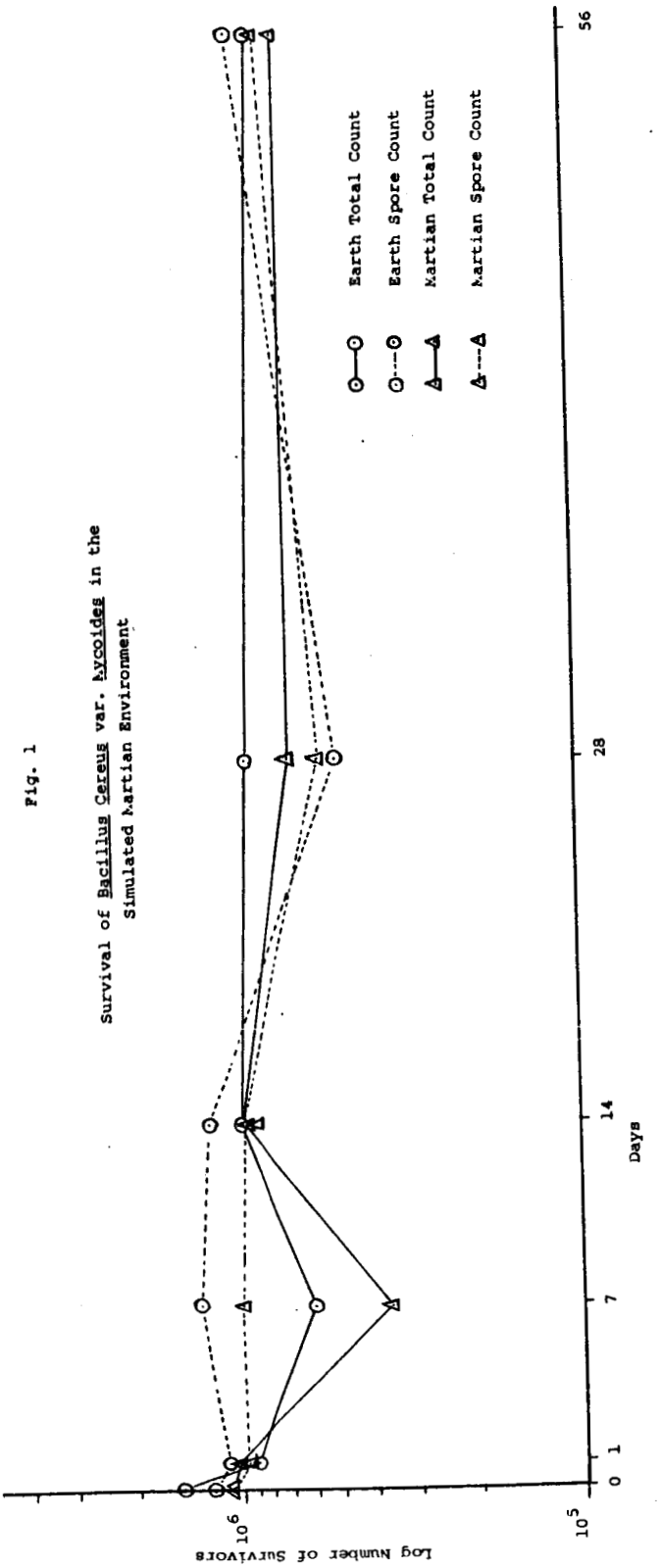

Fig. 2

The Effect of Two Percent Moisture on the Survival of Bacillus Subtilis var. Globigii in the Simulated Martian Environment

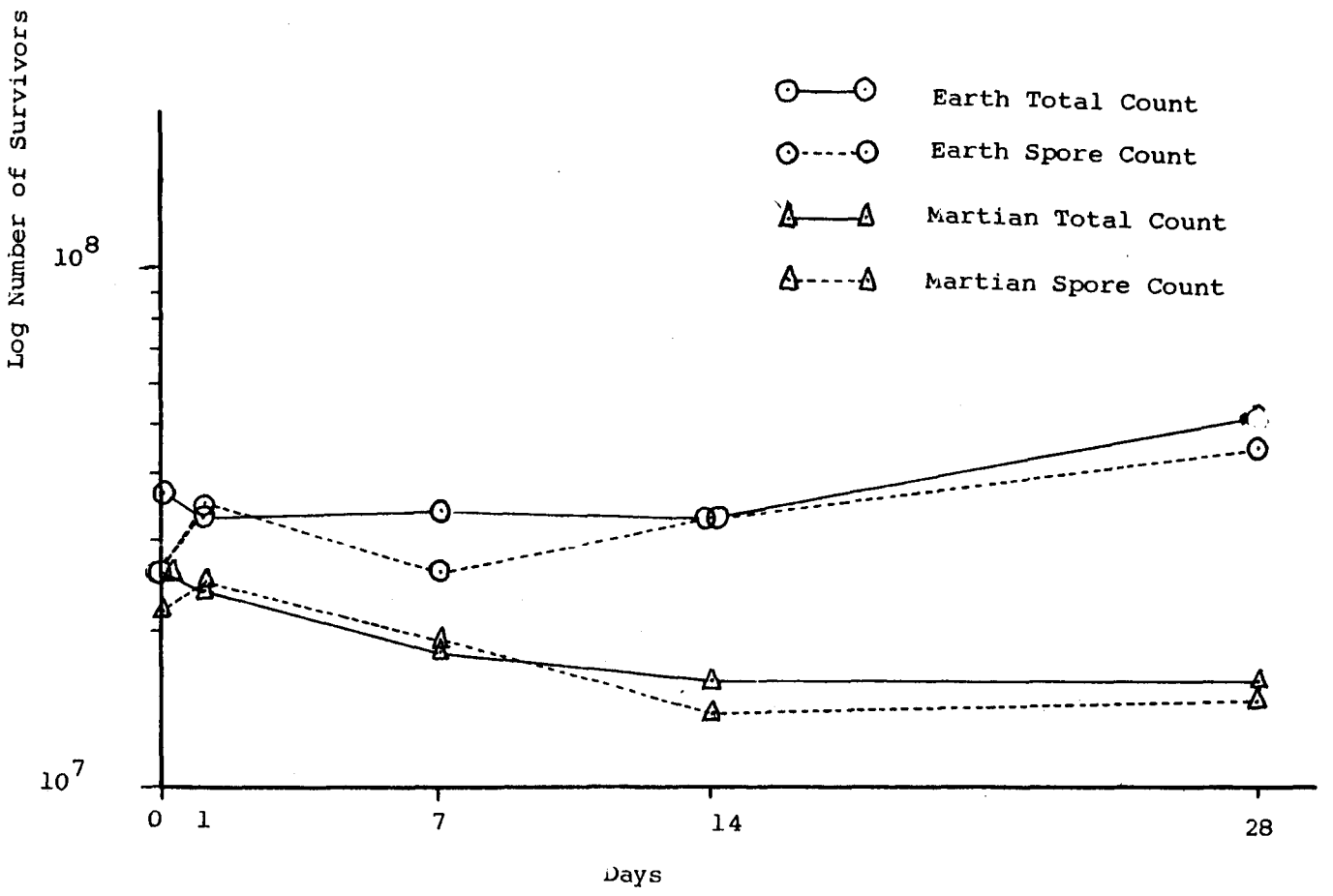

Table 5

THE EFFECT OF TWO PERCENT MOISTURE ON THE SURVIVAL OF BACILLUS SUBTILIS VAR. GLOBIGII IN THE SIMULATED MARTIAN ENVIRONMENT

	Number of Bacteria x 10^6/g*			
	Earth Control		Martian Experimental	
Days Exposure	Total Count**	Spore Count**	Total Count**	Spore Count**
0	37	26	26	22
1	33	35	24	25
7	34	26	18	19
14	33	33	16	14
28	51	45	16	15

*Inoculum/Tube: Total count 65 x 10^6
Spore count 37 x 10^6
% Spores 56.9

**Counts represent three samples plated in quadruplicate.

Fig. 3

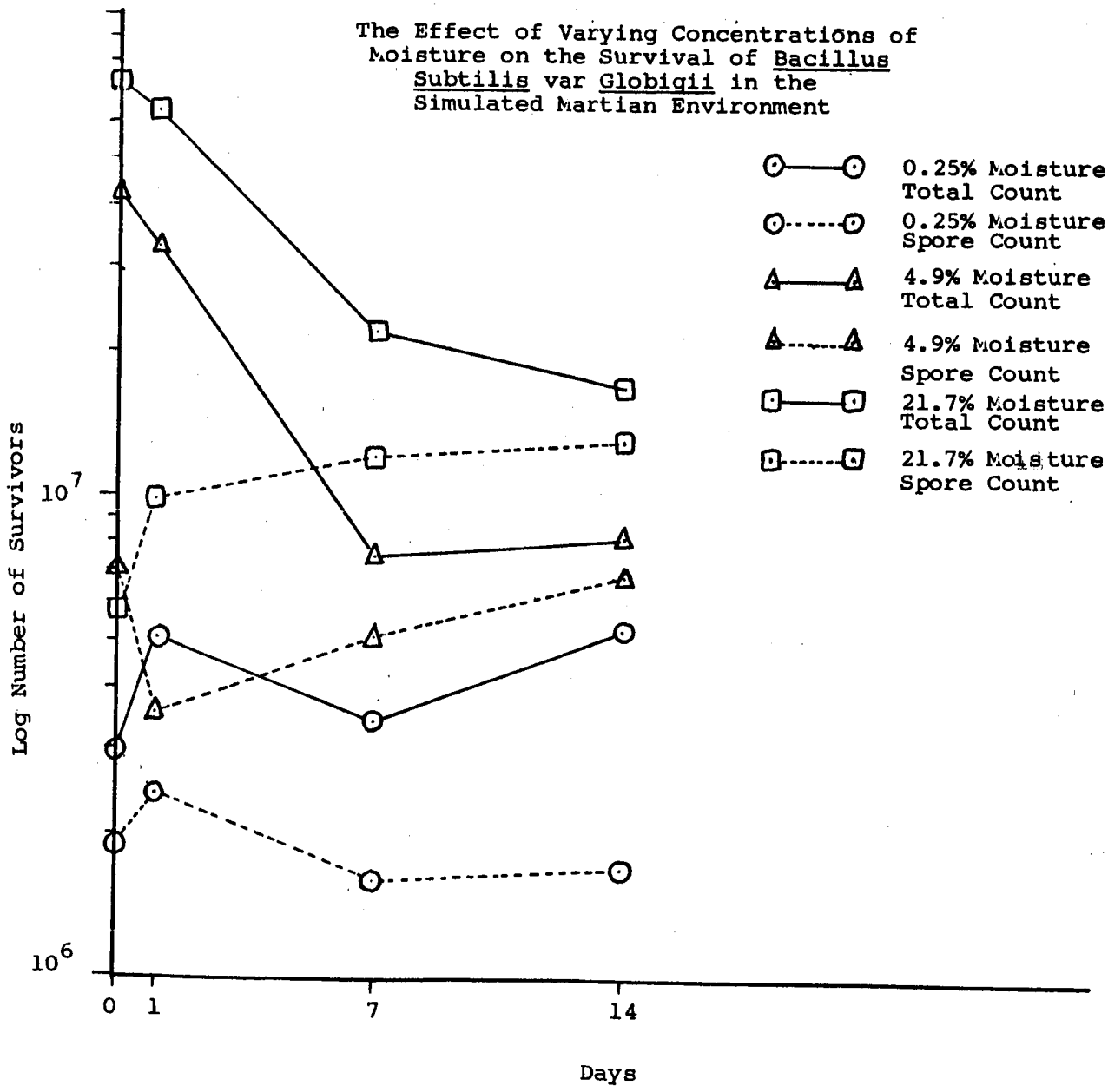
The Effect of Varying Concentrations of Moisture on the Survival of Bacillus Subtilis var Globigii in the Simulated Martian Environment
0.25% Moisture Total Count
0.25% Moisture Spore Count
4.9% Moisture Total Count
4.9% Moisture Spore Count
21.7% Moisture Total Count
21.7% Moisture Spore Count
Log Number of Survivors
10^7
10^6
0
1
7
14
Days

Table 6

THE EFFECT OF VARIOUS MOISTURE CONCENTRATIONS ON THE SURVIVAL OF BACILLUS SUBTILIS VAR. GLOBIGII IN THE SIMULATED MARTIAN ENVIRONMENT

	Number of Bacteria x 10^6/g*					
	0.25%		4.90%		21.7%	
Days Exposure	Total Count**	Spore Count**	Total Count**	Spore Count**	Total Count**	Spore Count**
0	3	1.9	41	7.1	72	5.8
1	5.1	2.4	33	3.6	63	9.8
7	3.4	1.6	7.5	5.1	22	12.1
14	5.3	1.7	8.2	6.8	17	12.8

*Inoculum/tube:

	0.25%	4.9%	21.7%
Total Count	92 x 10^6	58 x 10^6	80 x 10^6
Spore Count	51 x 10^5	66 x 10^5	66 x 10^5
% Spores	5.6	11.4	8.3

**Counts represent three samples plated in quadruplicate.

content and the high initial death rates as shown by the total count curves. The total number of spores and the rate of increase in the spore counts are a function of soil moisture. These studies are continuing and this point will be further substantiated in subsequent experiments.

Studies with lichens have been initiated to determine the ability of lichenized fungi to survive in the Martian atmosphere. Preliminary attempts to isolate the fungal derivative from the lichen have produced encouraging results. Germination, with moderate mycelial growth, from polyspore cultures of ascospores from lichen apothecia have occurred in the simulated Martian atmosphere. This is of interest since fungi have not been commonly observed to grow in anaerobic environments. Similar results, i.e., growth in simulated Martian environment, have been obtained with cultures of isidia, or marginal portions of lichen thallus. Subcultures of lichen fungi appeared to grow more vigorously in the simulated Martian atmosphere than subcultures in Earth atmosphere. Some pigment production has also occurred in lichenized fungi grown in the simulated Martian environment.

IV. SUMMARY

Bacterial counts are given for soil samples collected at Rocky Mountain National Park, (Colorado) and White Sand National Monument (New Mexico).

Facultative, lecithinase positive microorganisms, the majority of which are Gram-positive sporulating bacilli, have been isolated from a variety of desert and tundra soil samples. With few exceptions these bacteria are able to survive the simulated Martian environment.

Incorporation of different concentrations of moisture to the unusually dry simulated Martian soil (modified by adding 1% organic medium) increased the number of B. subtilis var globigii surviving the inoculating and flushing procedures. The overall effect of moisture on survival and growth of this organism in the simulated Martian environment is presently under study.

Fungal components of lichen species have been isolated and are able to survive the Martian atmosphere. Apothecia germination, with mycelial growth has also occurred in the simulated Martian environment. Pigmentation of fungal component while in the Martian atmosphere has been noticed.

V. FUTURE WORK

The future program will include:

1. Survival, adaptation, and growth studies of micro-organisms.
2. Survival, adaptation, and growth studies of plants
3. Biochemical studies relating to growth of micro-organisms and plants
4. Physical studies in support of the growth experiments.

The survival of a variety of bacteria in the simulated Martian environment has been demonstrated. The determination of what change(s) in the simulated Martian environment will enable the bacteria to grow must now be established. A critical examination of the function of moisture on the growth of selected strains of bacteria will be conducted using constant and diurnal temperatures of varying durations, the growth of the bacteria will be noted in soils containing different concentrations of moisture. Supportive physical methods e.g., methods for accurately determining small amounts of moisture will, necessarily, be developed.

The studies with plants will be similar in nature to those described for bacteria: establishment of the minimal environment required by selected plants for survival and growth.

Current studies with lichens will be continued. Growth of the fungal component in the anaerobic simulated Martian atmosphere is of chief interest. The indices of fungal growth will include apothecia germination, mycelial growth, and pigment production.

A detailed discussion of these proposed studies was presented in IITRI Proposal No. 64-91L, dated October 18, 1963.

VI. RECORDS AND PERSONNEL

The experimental data are recorded in Logbooks C13783, C13795, and C14081.

Technical assistance was given by Mr. John Rush and Miss Charlene Berger.

Respectfully submitted,

IIT RESEARCH INSTITUTE

Charles A. Hagen
Associate Bacteriologist
Life Sciences Research

Regnal Jones [1963] 0 refs
Assistant Biologist
Life Sciences Research

Approved by:

E. J. Hawrylewicz
Assistant Director
of Life Sciences Research

CAH/RJ/lp

Copy No._____

Distribution List:

Copy No.	Recipient
1 - 25	Office of Grants and Research Contracts Office of Space Sciences National Aeronautics and Space Administration Washington 25, D.C. Attention: SC
26	IIT Research Institute Division L Files
27	IIT Research Institute Editors, G. S. Gordon, Main Files
28	IIT Research Institute K. W. Miller, Report Library
29	Dr. Irving Davis Major, USAF, MSC European Office Office of Aerospace Research Shell Building, 45 Cantersteen Brussels, Belgium
30	Dr. C. S. Pittendrigh Department of Biology Princeton University P.O. Box 704 Princeton, New Jersey
31	Dr. Allan Brown Department of Botany University of Minnesota Minneapolis, Minnesota

Copy No.	Recipient
32	Dr. E. C. Polard Visiting Professor of Biophysics College of Chemistry and Physics Pennsylvania State University University Park, Pennsylvania
33	Dr. Norman Horowitz Biology Department California Institute of Technology Pasadena, California
34	Dr. Kirby-Smith Biology Division Oak Ridge National Laboratory P.O. Box Y Oak Ridge, Tennessee
35	Dr. Melvin Calvin Space Sciences Laboratory University of California Berkeley 4, California
36	Dr. Sidney Fox Florida State University Tallahassee, Florida
37	Dr. Carl Sagan Department of Astronomy University of California Berkeley, California
38	Dr. Dale Smith Ames Research Center National Aeronautics and Space Administration Moffett Field, California
39	Dr. Dale Jenkins Office of Space Sciences National Aeronautics and Space Administration Washington 25, D.C.
40	Dr. R. E. Cameron Jet Propulsion Laboratories California Institute of Technology Pasadena, California

25

NASA-22

4247300

N64 11293 *

IIT RESEARCH INSTITUTE

FORMERLY ARMOUR RESEARCH FOUNDATION OF ILLINOIS INSTITUTE OF TECHNOLOGY

Technology Center • 10 W. 35th Street, Chicago 16, Illinois • Area Code 312—225-9600

November 27, 1963

Office of Grants and Research
Contracts
Office of Space Sciences
National Aeronautics and Space
Administration
Washington 25, D. C.

next p.

Attention: SC

Gentlemen:

An error resulted in Report No. IITRI-3194-11 from using nonphotosensitive ink to mark "less than" symbols.

Errata: Table 4, p. 8

The total and spore counts for culture No. 21 at 0 and 28 days should read $<10^3$ instead of 10^3. The total and spore counts for culture No. 63 at 0 and 28 days should read 43×10^3, $<10^3$, and $<10^3$ instead of 43×10^3, 10^3, and 10^3.

Very truly yours,

Charles A. Hagen

Charles Hagen
Associate Bacteriologist
Life Sciences Research

CH/ct

RECEIVED
DEC 3 3 15 PM '63
OFFICE OF GRANTS & RESEARCH CONTRACTS