Report No. IITRI-L6032-11
(Quarterly Status Report)

LIFE IN EXTRATERRESTRIAL ENVIRONMENTS

Contract No. NASr-22

National Aeronautics and
Space Administration
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IIT RESEARCH INSTITUTE

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I. INTRODUCTION

Studies of the survival of Bacillus cereus and Staphylococcus aureus in severe environments for periods as long as 12 months are in progress. The following generalizations of the results to date can be made:

(1) B. cereus

(a) In earth atmospheres at reduced barometric pressures, growth occurred at 98 mb with a 20-hr daily freeze and at 10, 25, and 40 mb with an 8-hr daily freeze, but not at 10, 25, and 40 mb with a 20-hr daily freeze. At 1 year the populations were equal to or greater than the initial populations of 10^5 to 10^6 cells per gram of soil.

(b) In 37, 67, and 100% carbon dioxide atmospheres at barometric pressures from 5 to 98 mb with a 20-hr daily freeze, no growth occurred. At 1 year the populations were equal to the initial populations of 10^5 to 10^6 cells/g.

(2) S. aureus

(a) In earth atmospheres at reduced (5-, 10-, 25-, 40-, and 98-mb) barometric pressures with a 20-hr daily freeze, the final populations were 1 to 2 logs higher than the initial populations of 10^5 cells/g.

(b) In 36, 67, and 100% carbon dioxide atmospheres at barometric pressures from 5 to 98 mb with a 20-hr daily freeze, the final populations were 1 to 2 logs higher than the initial populations of 10^5 cells/g.

Studies on the effect of a low water activity (a_w) on S. aureus are in progress. Barometric pressure and length of daily freeze influenced the growth of S. aureus. Growth occurred in a 67% carbon dioxide atmosphere at 25 mb and an a_w of 0.81 to 0.85 with a 16-hr but not a 20-hr daily freeze. Growth also occurred in the same environment with an a_w of 0.70 to 0.75, but not with the same a_w in a 100% carbon dioxide atmosphere at 5 or 10 mb.

Soil ecology experiments on the growth responses of microorganisms in different soils in a simulated Martian environment (67% carbon dioxide at 15 mb and a 16-hr daily freeze) are in progress. These studies, related to the probability of extraterrestrial contamination, will evaluate the minimum a_w and the numbers of the following microorganisms required for growth and survival: B. cereus, Lactobacillus plantarum, Pseudomonas aeruginosa, Putrefactive Anaerobe (PA 3679), S. aureus, and Streptomyces albus.

Preliminary data indicate that the a_w of the environment influences the minimum number of organisms required for survival. For example, L. plantarum survived in a brunizem soil (neutral pH and moderate organic content) with an initial inoculum of one cell/g at an a_w of 0.92 and with an initial inoculum of 100 cells/g at an a_w of 0.78.

Studies with the Martian chamber were started. Initial experiments with a brunizem soil will investigate temperature as a function of soil depth and the formation of a permafrost layer with daily freeze-thaw cycles.

II. EXPERIMENTAL PROCEDURES

Stock culture preparations of the microorganisms were described in Reports No. IITRI-L6023-5, -6, and -7. For the soil ecology experiments, the preparation and the inoculation of test tubes and the methods for determining the a_w of the microorganisms were described in Report No. IITRI-L6023-9.

All stock culture cell suspensions were stored at 4°C until used. B. cereus and PA 3679 spore suspensions were heat-shocked at 80°C for 10 min just before use.

Some of the soil ecology experiments started during the last report period utilized the following environmental conditions:

(1) Gaseous composition: 67% carbon dioxide, 30% nitrogen, and 3% argon

(2) Atmospheric pressure: 15 mb

(3) Temperature cycle: 16-hr daily freeze at -65°C and 8-hr daily thaw at 25°C

(4) Moisture concentration: maximum to minimum a_w, depending on the organism's requirement

(5) Soil type: brunizem, desert, and podzol

(6) Organism: B. cereus, L. plantarum, PA 3679, P. aeruginosa, S. aureus, and Streptomyces albus

(7) Cell concentration: usually 10^3, 10^2, and 10 cells/g.

The two lowest numbers of cells and the minimum a_w are studied for survival and growth in the environment over a 56-day test period. Bacterial counts of viable cells are made at 7, 28, and 56 days and are compared to initial counts. The bacterial counts are reported as averaged counts of two plates from each of three or five tubes (depending on the experiment). Incubation was at 35, 30, or 25°C for 1 to 5 days, depending on the bacterial species.

III. RESULTS AND DISCUSSION

In experiments that investigated the effects of barometric pressure, gaseous composition, moisture concentration, and daily freeze-thaw cycles on B. cereus and S. aureus, sufficient replicates were prepared that results on the survival and the growth of these organisms could be obtained past 56 days. The data are shown in Tables 1 and 2.

Growth of B. cereus occurred in an earth atmosphere at 98 mb with a 20-hr daily freeze but did not occur when the barometric pressure was lowered to 40, 25, or 10 mb. The pressure and the length of the daily freeze appear to be factors regulating

Table 1

SURVIVAL OF *B. CEREUS* IN DIFFERENT SEVERE ENVIRONMENTS

Environmental Conditions[a]				Viable Cells/g of Soil x 10^5				
				Total Count		Spore Count		
Gaseous Composition	Barometric Pressure, mb	Daily Freeze, hr	Time in Environment, months	Initial	Final	Initial	Final	Growth[b]
Earth atmos.	98	20	12	3.4	6.9	2.2	2.0	+
	40	20	6	3.0	4.1	1.8	1.8	0
	25	20	12	3.2	0.6	1.5	0.7	0
	10	20	12	2.9	3.7	1.6	1.5	0
37% CO_2	98	20	6	3.0	19.0	2.1	2.9	±
	40	20	12	2.5	3.4	2.1	0.5	0
67% CO_2	98	20	12	2.9	0.9	1.8	0.8	0
	25	20	12	3.1	1.2	2.5	1.0	0
	10	20	2	2.6	3.5	1.2	0.9	0
	10	16	2	2.6	4.8	1.2	0.8	+
	5	20	2	2.1	1.0	0.9	0.8	0
	5	16	2	2.1	2.1	0.9	0.7	0
100% CO_2	98	20	12	2.9	1.1	2.2	0.9	0
	10	20	6	3.1	2.4	2.5	0.9	0

[a] The a_w was between 0.96 and 0.99.

[b] Growth was recorded as positive when the viable cell count increased 10-fold above the initial count at some time during the experiment.

Table 2

SURVIVAL OF S. AUREUS IN DIFFERENT SEVERE ENVIRONMENTS

Environmental Conditions[a]				Viable Cells/g of Soil x 10^5 Total Count		
Gaseous Composition	Barometric Pressure, mb	Daily Freeze, hr	Time in Environment, months	Initial	Final	Growth[b]
Earth atmos.	98	20	2	7.5	3000	+
	40	20	6	1.5	40	+
	25	20	6	0.3	22	+
	10	20	12	0.3	14	+
	5	20	2	1.1	60	+
37% CO_2	98	20	12	5.2	31	+
	40	20	12	1.2	6	+
67% CO_2	98	20	6	4.5	32	+
	25	20	2	2.9	130	+
	10	20	2	6.1	11	+
	10	16	2	6.1	67	+
	5	20	2	6.3	30	+
	5	16	2	6.3	83	+
100% CO_2	98	20	6	7.2	46	+
	10	20	12	2.4	10	+
	5	20	2	3.1	55	+

[a] The a_w was between 0.95 and 0.99.

[b] Growth was recorded as positive when the viable cell count increased 10-fold above the initial count at some time during the experiment.

the growth of this organism. Previous experiments with an 8-hr instead of a 20-hr daily freeze permitted the organism to grow. Growth also occurred in a 67% carbon dioxide atmosphere at 10 mb with a 16-hr daily freeze, but not with a 20-hr daily freeze.

An attempt to relate the growth response of B. cereus to the partial pressure of carbon dioxide was not possible because of insufficient data. Actually, three factors are involved: barometric pressure, carbon dioxide concentration, and length of daily freeze. The barometric pressure exerts a double effect by influencing the a_w of the system as well as the carbon dioxide concentration. For example, with a 67% carbon dioxide atmosphere, the a_w requirement of an organism may be satisfied with a 98-mb pressure, but the elevated pressure would increase the concentration of carbon dioxide to a toxic level.

In a similar manner, an organism may grow in a suboptimal environment with a daily freeze of 16 hr but not 20 hr. All the environments in these experiments were suboptimal, and the interactions of the factors that make up the environments and the values of those factors that limit the growth of various organisms are being investigated.

The most significant finding from the data in Table 1 is that B. cereus survives for long periods of time in many kinds of environments with little loss of viability. Initial

populations were between 10^5 and 10^6 cells/g and were similar to those recorded after as long as 12 months in the environment. This result has serious implications in planetary contamination, since repeated contamination would be cumulative even in the absence of growth.

S. aureus grew in all the environments (Table 2). The final viable cell counts were always higher than the initial counts, even after 12 months in the environments. There was an indication that the final populations were higher with less severe environmental conditions, although the difference may be the result of a slower growth response to the more severe environments.

The effect of low a_ws on S. aureus is shown in Table 3. An effect of barometric pressure and length of daily freeze on growth was noticed. S. aureus grew in a 67% carbon dioxide atmosphere at 25 mb and an a_w of 0.81 to 0.85 with a 16-hr but not a 20-hr daily freeze, a result that was a temperature effect. Growth occurred in the same environment with an a_w of 0.70 to 0.75 and a 16-hr daily freeze, but not with the same a_w in a 100% carbon dioxide atmosphere at 5 or 10 mb with a 16-hr daily freeze. The growth inhibition, or delayed growth response, was caused by the decrease in barometric pressure, which lowered the a_w and not the toxicity from the carbon dioxide. The carbon dioxide concentration was greater with

the 67% carbon dioxide atmosphere at 25 mb than with the 100% carbon dioxide atmosphere at 10 or 5 mb.

Table 3

EFFECT OF REDUCED a_w ON GROWTH OF S. AUREUS IN SEVERE CARBON DIOXIDE ENVIRONMENTS

Environmental Conditions				
CO_2 Content, %	Barometric Pressure, mb	Daily Freeze, hr	a_w Range	Growth
67	25	20	0.86-0.90	+
	25	16	0.81-0.85	+
	25	20	0.81-0.85	0
	25	16	0.70-0.75	+
100	10	16	0.86-0.90	+
	10	16	0.70-0.75	0
	5	16	0.86-0.90	+
	5	16	0.70-0.75	0

The growth of S. aureus at a_ws lower than those previously reported (Scott, W. J., Australian J. Biol. Sci., Vol. 6, pp. 549-564, 1953) was caused by the daily freeze-thaw cycles. A similar phenomenon was reported for spore germination and growth of B. cereus and B. subtilis (Hagen C. A., et al, Appl. Microbiol., Vol. 15, pp. 285-291, 1967). Contrary to popular belief, daily freeze-thaw cycles are not necessarily lethal

to an organism, but actually allow growth to take place at lower a_ws than would be possible at constant above-freezing temperatures.

Preliminary data from the soil ecology experiments indicate that survival of an organism in a particular soil depends on the initial numbers present and the a_w of the environment, which is 67% carbon dioxide at 15 mb with a 16-hr daily freeze.

L. plantarum survived in a brunizem soil (neutral pH and moderate organic content) with an initial inoculum of 1 cell/g at an a_w of 0.92 and with an initial inoculum of 100 cells/g at an a_w of 0.78. Growth of the organism occurred with an initial inoculum of 10 cells/g and an a_w of 0.95. S. aureus responded in a similar manner. An inoculum of 10 cells/g survived with an a_w of 0.94 but not with an a_w of 0.91, and an inoculum of 100 cells/g survived with an a_w of 0.89. B. cereus survived with initial inocula of 10 to 100 cells/g with a_ws from 0.91 to 0.94.

L. plantarum survived and grew in podzol soil (acid pH and low organic content) with initial inocula of 1 to 1,000 cells/g with an a_w of 0.95. An initial inoculum of 100 cells/g survived with an a_w of 0.91 but not with an a_w of 0.87. And 10 cells/g, but not 1 cell/g, survived with an a_w of 0.93. B. cereus survived with an initial inoculum of 10 cells/g with an a_w of 0.91.

These studies will be completed and the results will be presented in the next report.

Studies with the Martian chamber were started during this report period. A brunizem soil is being used initially to test the soil-coring tool and other experimental factors, such as rate of freezing with soil depth, effect of daily freeze-thaw cycles on the temperature gradient of the soil profile, and requirements to establish a permafrost layer.

IV. SUMMARY

B. cereus and S. aureus survived in various severe environments for at least 12 months with no appreciable decrease in the number of viable cells. Barometric pressure, carbon dioxide concentration, and length of daily freeze influenced the growth response of both organisms.

One of the most severe environments tested, 100% carbon dioxide at 10 mb with a 20-hr daily freeze, allowed survival with no appreciable die off of B. cereus. Growth of S. aureus occurred in the same environment, with final counts about 4-fold higher than initial counts. The importance of B. cereus survival and S. aureus survival and growth to extraterrestrial planetary contamination is evident.

Barometric pressure and length of daily freeze affected the growth of S. aureus in environments with low a_ws. The lowest a_w range thus far tested that permitted growth was 0.70 to 0.75.

Preliminary data from soil ecology experiments indicated that the a_w of the environment is important to survival. As the a_w decreased, a greater number of cells was required for survival. For example, 1 cell/g of L. plantarum survived in a brunizem soil at an a_w of 0.92, but 100 cells/g were required for survival at 0.78 a_w. Growth occurred with an initial inoculum of 10 cells/g and an a_w of 0.95.

Studies with the Martian Chamber were started. Initial studies are examining environmental factors such as rate of freezing with soil depth, effect of daily freeze thaw cycles on the temperature gradient of the soil profile, and requirements to establish a permafrost layer.

V. PERSONNEL AND RECORDS

The experiments were planned with the counsel of Dr. E. J. Hawrylewicz and the technical assistance of Mr. Bruce Anderson, Mrs. Marjorie Cephus, and Miss Vivian Tolkacz.

Experimental data are recorded in IITRI Logbooks C16684, C16876, C16889, C16938, C17092, C17094, C17260, C17271, C17272, C17497, C1759e, C17605, C17587, C17849, C18029, C18161, and C18169.

Respectfully submitted,

IIT RESEARCH INSTITUTE

Charles A. Hagen
Research Bacteriologist
Life Sciences Research

Approved by:

E. J. Hawrylewicz
Assistant Director
Life Sciences Research

CAH/cg

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