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**STUDIES WITH A SIMULATED MARTIAN
ENVIRONMENT**

Bacterial Synergism: Preliminary Systems

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**USAF School of Aerospace Medicine
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FOREWORD

This report was prepared by the following personnel in the Astrobiology Branch:

THOMAS L. ROBERTS, M.S.

ROBERT J. BALL, JR., B.S.

E. STATEN WYNNE, Ph.D.

ABSTRACT

Simulated Martian environment permitted survival of endospores of *Bacillus cereus* but not the photosynthetic nitrogen-fixing soil bacterium, *Rhodospirillum rubrum*. There was no evidence of a synergistic relationship. Observed increases in colony counts of *B. cereus* appeared due to temperature cycling.

This technical documentary report has been reviewed and is approved.


ROBERT B. PAYNE
Colonel, USAF, MSC
Chief, Operations Division

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STUDIES WITH A SIMULATED MARTIAN ENVIRONMENT

Bacterial Synergism: Preliminary Systems

1. INTRODUCTION

Kooistra et al. (5) reported that when they used microflora of native soil in a simulated Martian environment, the microflora survived; moreover, the bacteria in the microflora increased in numbers. These results were confirmed by Davis and Fulton (2) with an unidentified strain of *Bacillus cereus*. Other studies by Roberts and Wynne (6) also indicated increased counts of *B. cereus*.

Since some microorganisms survived and appeared to multiply in simulated Mars environment, it was reasoned that photosynthetic, nitrogen-fixing bacteria might survive and have potential agricultural usefulness in colonizing Mars. Except for its donor requirements of biotin and organic hydrogen, *Rhodospirillum rubrum*, a bacteriochlorophyll-containing heterotroph that utilizes carbon dioxide as sole carbon source, might photosynthesize and fix nitrogen in such an environment.

Under suitable conditions, certain organisms may limit or extend the survival of others. The characteristics of *R. rubrum* (3) suggested suitability for synergism with *B. cereus*, which conceivably could furnish hydrogen donor and biotin requirements of *R. rubrum*. The purpose of this paper is to report studies on survival of *R. rubrum* under simulated Martian conditions, as well as effects of temperature cycling on these two potential synergists.

2. SUMMARY

Evidence has been presented to show that increases in colony counts of *Bacillus cereus*

under simulated Martian environment are due to temperature cycling. *Rhodospirillum rubrum* was unable to survive 48 hours under these conditions, and there was no evidence of synergism. The Martian regimen was without effect on moisture content of the soil employed.

3. MATERIAL AND METHODS

R. rubrum 11170 (ATCC) was cultured in Hutner's medium (4) for 7 days at room temperature; *B. cereus* was cultured in nutrient broth at 37° C. for 20 to 30 hours. Following incubation the cultures were harvested, washed, and resuspended in 2.2 ml. water. Bacteriologic staining of *B. cereus* showed spore formation in at least 90% of the cells. Aliquots of cultures of each organism were placed in separate 15 gm. portions of sterilized dry, red sandstone (2), and aliquots of the two cultures were combined in additional 15 gm. portions of the soil. Hutner's medium was also inoculated with *R. rubrum* for control purposes. All inoculated soil was dried in a vacuum desiccator at room temperature, mixed by shaking, distributed in 0.13 gm. portions in glass tubes, and placed in "Mars jars" (2). Simulated Martian environment in the "Mars jars" was established by evacuating, flushing several times with moisture-free nitrogen, and filling with a gaseous mixture containing 93.54% nitrogen, 4.24% argon, 2.21% carbon dioxide, and 0.01% oxygen, to a pressure of 65 mm. Hg (1). Temperature and light were regulated to give a nocturnal-diurnal cycle of 16 hours' darkness at -25° C. followed by 8 hours light at 22° to 25° C. Controls were run at normal temperature and pressure, and also with temperature cycling at ambient atmosphere (table I).

TABLE I
Combinations of bacteria and individual physical factors

Jars		Physical factors		Bacterial species	
Series	Number	Environment*	Temperature cycling	<i>B. cereus</i>	<i>R. rubrum</i>
I	3	Martian	+	+	-
II	3	Martian	+	-	+
III	3	Martian	+	+	+
IV	3	Earth	+	+	-
V	3	Earth	+	-	+
VI	3	Earth	+	+	+
VII	3	Earth	-	+	-
VIII	3	Earth	-	-	+
IX	3	Earth	-	+	+

*Pressure and gases.

TABLE II
The response to Martian environmental factors of R. rubrum in Hutner's medium

Environment*	Temperature cycling	Colony count				
		Day 0	Day 1	Day 2	Day 4	Day 8
Martian	+	1×10^4	5.4×10^3	1×10^2	None	None
Earth	+	9.6×10^3	9.7×10^2	5×10^2	None	None
Earth	-	1.3×10^4	1×10^5	1.4×10^6	1×10^8	9.7×10^6
Martian	-	1×10^4	5.4×10^4	9.6×10^5	1×10^7	1×10^7

*Pressure and gases.

R. rubrum in Hutner's medium was subjected to conditions shown in table II.

On days 0, 1, 2, 8, 15, 30, and 45, three tubes were removed from one jar in each series (table I) for bacterial counts and moisture determination. On day 4, tubes from jars in series I, II, and III were analyzed. In all series, the jars were sampled in rotation, and returned to the experimental conditions without delay. Prior to entry, the internal pressure of the jars was verified to be approximately 65 mm. Three aliquots were removed from each jar, and triplicate platings were made from serial dilutions of each aliquot. Bacterial counts were done in nutrient agar or Hutner's agar medium. Moisture determinations were made on aliquots in moisture-free, 7-ml. screw-cap vials. After weighings, the

vials were dried at 105° C. for 48 hours, reweighed, emptied of soil, and weighed again.

4. RESULTS AND DISCUSSION

In all series involving temperature cycling, *S. cereus* showed comparable and statistically significant ($P < .01$) increases in colony counts with time (table III, series I, III, IV, and VI). Without temperature cycling, no significant changes in colony counts of this organism occurred (series VII and IX). These findings corroborate previous reports (2, 6) of increased colony counts of spore formers in simulated Martian environments, but indicate that the increases may have been due solely to temperature cycling. Interruption of dormancy of spores, with subsequent germination, is suggested as an explanation.

TABLE III

Response of bacterial populations exposed to simulated Mars environmental factors in soil

Jar series	Factors	Bacteria	Day 0		Day 1		Day 2		Day 4		Day 8		Day 15		Day 30		Day 45		
			Colony counts	% M	Colony counts	% M	Colony counts	% M	Colony counts	% M	Colony counts	% M	Colony counts	% M	Colony counts	% M	Colony counts	% M	Colony counts
I	M	<i>B. cereus</i>	4.6 × 10 ⁸	0.36	4.7 × 10 ⁸	0.37	5.2 × 10 ⁸	0.37	6.2 × 10 ⁸	0.34	7.9 × 10 ⁸	0.37	7.9 × 10 ⁸	0.36	8.9 × 10 ⁸	0.33	9.4 × 10 ⁸	0.35	
III	M	<i>B. cereus</i> <i>R. rubrum</i>	4.5 × 10 ⁸ 3.5 × 10 ⁸	0.35	3.5 × 10 ⁸ 1.5 × 10 ⁶	0.37	6.1 × 10 ⁸ None	0.37	7.1 × 10 ⁸ None	0.36	7.9 × 10 ⁸ None	0.33	7.9 × 10 ⁸ None	0.34	9.1 × 10 ⁸ None	0.32	9.3 × 10 ⁸ None	0.35	
II	M	<i>R. rubrum</i>	3.4 × 10 ⁸	0.32	1.0 × 10 ⁶	0.36	None	0.37	None	0.36	None	0.35	None	0.32	None	0.34	None	0.33	
VIII	RT	<i>R. rubrum</i>	2.4 × 10 ⁸	0.35	3.0 × 10 ⁸	0.34	3.0 × 10 ⁸	0.37	3.0 × 10 ⁸	—	None	0.36	None	0.36	None	0.33	None	0.34	
VII	RT	<i>B. cereus</i>	3.5 × 10 ⁸	0.34	3.6 × 10 ⁸	0.35	3.2 × 10 ⁸	0.36	—	—	3.0 × 10 ⁸	0.34	3.1 × 10 ⁸	0.35	3.0 × 10 ⁸	0.36	3.0 × 10 ⁸	0.35	
IV	TC	<i>B. cereus</i>	3.5 × 10 ⁸	0.37	4.2 × 10 ⁸	0.35	5.9 × 10 ⁸	0.37	—	—	4.4 × 10 ⁸	0.37	5.1 × 10 ⁸	0.32	6.1 × 10 ⁸	0.24	6.9 × 10 ⁸	0.35	
V	TC	<i>R. rubrum</i>	3.5 × 10 ⁸	0.35	1.2 × 10 ⁶	0.36	None	0.37	—	—	None	0.35	None	0.34	None	0.35	None	0.34	
VI	TC	<i>B. cereus</i> <i>R. rubrum</i>	4.5 × 10 ⁸ 3.5 × 10 ⁸	0.35	4.0 × 10 ⁸ 1.6 × 10 ⁶	0.37	5.3 × 10 ⁸ None	0.37	—	—	7.8 × 10 ⁸ None	0.36	7.9 × 10 ⁸ None	0.37	8.5 × 10 ⁸ None	0.37	9.3 × 10 ⁸ None	0.36	
IX	RT	<i>B. cereus</i> <i>R. rubrum</i>	3.7 × 10 ⁸ 3.5 × 10 ⁸	0.34	3.6 × 10 ⁸ 3.1 × 10 ⁸	0.36	3.4 × 10 ⁸ 3.1 × 10 ⁸	0.35	—	—	3.1 × 10 ⁸ None	0.34	3.1 × 10 ⁸ None	0.35	3.0 × 10 ⁸ None	0.36	3.0 × 10 ⁸ None	0.36	

M, Mars gases, temperature, and pressure; RT, room temperature conditions; TC, room temperature conditions and temperature cycling; % M, percent moisture.

In all series involving temperature cycling, viability of *R. rubrum* declined rapidly, and this organism was not recovered on day 2 (table III, series II, III, V, and VI). Without temperature cycling, however, recoveries of this organism were unaffected for at least 4 days (series VIII). Controls in Hutner's medium showed qualitatively similar results with temperature cycling, although the decline was somewhat less rapid (table II); *R. rubrum* was apparently

unaffected by uncycled simulated Martian environment (table II, last line).

It is obvious from the above findings that *B. cereus* and *R. rubrum* showed no evidence of synergism and, therefore, do not appear promising for colonization of Mars.

No statistically significant change in moisture content of the soil during exposure to simulated Mars environment was found.

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