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SURVIVAL OF SELECTED MICROORGANISMS IN HIGH ULTRAVIOLET FLUX

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INTRODUCTION

One argument against the hypothesis that life exists on Mars is that the action of ultraviolet radiations on terrestrial organisms is often lethal (1, 2, 3). Whereas the ozone layer in the earth's atmosphere prevents a significant amount of ultraviolet from reaching the earth's surface, no such protective layer exists on Mars (4, 5, 6). Therefore, the Martian surface would receive approximately 90 percent of the ultraviolet radiation between 2000 and 3000 Å incident on the planet (4). This surface flux is calculated to be approximately 80 $\mu a/cm^2$ (7).

The literature contains many studies on the lethal action of UV on bacteria (8, 9, 10, 11, 12, 13). Little is available on the survival of fungi, although in the last decade mutation studies on fungi, yeasts, and bacteria, using UV as the mutagen, have been reported (14, 15, 16, 17, 18, 19, 20).

The wavelength range of 2600 Å ± 100 Å is reported to be the most effective for producing both lethal and mutagenic effects in microorganisms (1, 2, 14).

The work reported here was an attempt to survey the survival times of selected microorganisms exposed to UV radiation in doses at a flux equivalent to that received on the Martian surface.

MATERIALS AND METHODS

Exposure Parameters

Model PF 516 ultraviolet lamps* with 98-percent radiation output at 2537 Å were set up in a plastic enclosure 14 inches from the sample exposure surface. Intensity of the radiation, measured by a GE Germicidal UV Intensity Meter #C, was approximately 80 $\mu w/cm^2$ at a distance of 14 inches.

*Atlantic Ultraviolet Corporation, New York.

Tests were performed at room temperature, approximately 23°C.

Organisms Used

Table 1 lists the organisms studied, with their optimal growth temperatures and the growth media used. Bacteria, actinomycetes, fungi, and one yeast are included.

Table 1
Organisms Studied

Organism	Optimum Temperature (°C)	Growth Media Used*
Bacillus subtilis var. niger	37	E
Brevibacterium helvolum**	25	E
Nocardia asteroides**	37	E
Streptomyces griseus**	37	E
Rhodotorula glutinis**	25	PDA; Sab
Aspergillus niger**	25	PDA; Sab
Cladosporium resinae**	25	PDA; Sab
Alternaria brassicola**	25	PDA; Sab
Penicillium notatum**	25	PDA; Sab
Hormodendrum hordei	25	PDA; Sab

*E = Eugonagar (Baltimore Biological Laboratories); PDA = potato dextrose agar (Fisher Scientific); Sab = Sabouraud dextrose agar (Fisher Scientific)
**Genera collected on balloon flights

All the organisms chosen except Bacillus subtilis var. niger and Hormodendrum hordei are species of genera collected by V. W. Green (21) on balloon flights to 20-27 km.

Growth of the fungi on potato dextrose agar (PDA) slants for 4 - 7 days resulted in profuse spore formation in all cases except Alternaria brassicola, which produced few spores. The yeast was also grown on PDA, for 3 days. Bacteria and actinomycetes were grown for 1 - 4 days on Eugonagar.

Each organism was harvested by scraping spores or vegetative growth off the slant into sterile distilled water. This suspension was removed to a clean, sterile tube, and dispersed by 30-sec agitation on a "Genie" vortex mixer. The gross particles were allowed to settle out; then the upper, relatively homogeneous layer was pipetted off for use.

Concentration

In the first series of experiments, the approximate concentration of each organism in a distilled water suspension was determined visually.

The second series of experiments, using only the fungi, required a much more accurate knowledge of the approximate cell concentration. Counts made on a suspension in 1% NaCl, using a Coulter Model B electronic particle counter with a 100μ aperture tube and threshold settings 8-∞, were checked against standard plate counts for accuracy. Correlation between the two counts was high (Table 2).

Table 2
Comparison of Fungal Concentrations
(Calculated from plate counts
and Coulter Counter readings)

Organism	Coulter Count (spores/ml)	Plate Count (spores/ml)
Aspergillus niger	1.16×10^7	0.76×10^7
	1.16×10^7	0.90×10^7
	6.04×10^6	4.65×10^6
Hormodendrum hordei	5.35×10^6	5.55×10^6
	5.35×10^6	4.97×10^6
	4.54×10^6	5.94×10^6
Cladosporium resinae	3.85×10^6	4.03×10^6
	3.85×10^6	3.60×10^6
Penicillium notatum	8.29×10^6	6.10×10^6
	8.29×10^6	8.30×10^6

Exposure Method

Cell suspensions of approximately 5000 cells per ml. were prepared in sterile distilled water; 0.2 ml. of each of these suspensions was spread on a plate of the appropriate agar medium (Eugonagar for bacteria and actinomycetes; Sabouraud dextrose agar for fungi and yeast) and allowed to dry, covered, at room temperature for about 30 minutes. Two plates of each organism were exposed to ultraviolet light in the plastic enclosure for each time interval desired. The plates were then incubated at the organism's optimum growth temperature for up to 7 days, with daily observations of the number, appearance, and size of colonies. (Weekend observations were not taken.)

Controls

Two plates of each organism were prepared as controls using the same procedure as for exposed plates. The controls were not exposed to ultraviolet radiation.

RESULTS

Tables 3, 4, and 5 list results of the first series of experiments to determine the approximate survival times of microorganisms. Times range from about 5 minutes or less for the bacteria and actinomycetes to more than 2 hours for one fungus, Aspergillus niger.

The initial results are qualitative rather than quantitative, as the exact concentration of the inoculum was unknown. After more accurate counting of the fungi with the Coulter counter, a second series of experiments was begun to quantitate the results. Table 5 includes these data (Series 2).

No end point (zero survivors) was found for any fungus except Penicillium notatum, Series 2 (10 minutes).

Three species of fungi exhibit a definite "plateau," the number of survivors remaining relatively constant for several time intervals. The plateau occurs at 30 - 60 minutes for A. niger, 15 - 30 minutes for C. resinae, and 20 - 40 minutes for A. brassicola. Data on H. hordei and P. notatum are too incomplete to provide even tentative conclusions, and only one run was performed with the actinomycetes and yeast.

Brevibacterium helvolum, on the other hand, exhibits a logarithmic type of death rate (Table 3). B. subtilis var. niger seems to exhibit traces of both

logarithmic death (Run 1; 0 - 90 sec, Runs 2 and 3; and 0 - 30 sec, Run 4) and the plateau (60 - 150 sec, Run 3; 90 - 150 sec, Run 4).

Table 3
Effect of Ultraviolet Radiation on Bacteria
(2537 Å, flux = ~ 80 $\mu\omega/cm^{-2}$)
(colonies per plate)

Exposure (sec)	Run 1	Run 2	Run 3	Run 4
Bacillus subtilis var. niger				
0	TNTC*	TNTC	67.5	45.0
30	178.0		3.5	39.5
60	123.5	37.5	1.0	21.0
90		27.0	1.0	2.0
120		20.5	0.5	4.0
150		7.0	0.5	3.0
180	4.0	3.5	0.0	0.5
300	2.0			
Brevibacterium helvolum				
0	TNTC	31.0	127.0	149.0
30	TNTC		74.0	36.5
60	TNTC	9.5	13.5	14.0
90		2.0	6.0	5.0
120		1.0	2.0	0.5
150		0.0	1.0	0.5
180	0.0	0.0	0.0	0.0
300	0.0			

*Too numerous to count

Table 4
Effect of Ultraviolet Radiation
(2537 Å) on Actinomycetes and Yeast
(colonies per plate)

Exposure (sec)	Nocardia asteroides	Streptomyces griseus	Rhodotorula glutinis
0	TNTC*	TNTC	TNTC
30	500.0	500.0	TNTC
60	135.5	350.0	TNTC
180	2.0	2.5	500.0
300	0.0	0.0	1.5

*Too numerous to count

DISCUSSION

The usual phenomenon observed in sterilization studies with microbes is a logarithmic order of death. The data presented here show that, in all but one case (Brevibacterium helvolum), this phenomenon is not observed, or only partially observed. Most of the other species exhibit a definite "plateau" where the number of survivors remains relatively constant. This phenomenon deserves further investigation.

The bacteria, actinomycetes, and yeast showed very short survival times, and no further work is planned to quantitate the results. The fungi, on the other hand, demonstrate substantial resistance to UV. Further study of the morphological and physiological characteristics of these organisms may make it possible to identify the protective mechanism(s). All the fungi studied are Deuteromycetes (Fungi Imperfecti).

Table 5
Effect of Ultraviolet Radiation (2537 Å) on Fungi

Exposure (min)	Aspergillus niger		Cladosporium resinae		Hormodendrum hordei		Alternaria brassicola		Penicillium notatum	
	Series 1	Series 2	Series 1	Series 2	Series 1	Series 2	Series 1	Series 2	Series 1	Series 2
0	+++	~1000*	+++	~1000*	+++++	~1000 1500*	++	~400*	+++ ++	~1000*
1	+++		+++		+++++		++		162, ~50	2, 5
5	++		++		++++		++		32	0
10	+		+		+++		++			
15	~50		54, 25	5, 6	++	+, 108, 87	+, 100			
20			23				85			
25			22				86			
30	~25, 38	30, 31	18, 15	1	+	48, 11, 20	89, 89	205		
40	~25		7				82			
45						9, 7				
50	~25		1				70			
60	~25, 18	7, 9	1			5,8	77	125		
80	28									
100	18									
120	7									

+ The number of + indicates the relative density of colonies. Actual count not determined (too numerous)

* Calculated from counts made on a dilution

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