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The Survival of Mesophilic and Thermophilic Bacteria Subjected to UV Irradiation

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THE SURVIVAL OF MESOPHILIC AND THERMOPHILIC BACTERIA SUBJECTED TO UV IRRADIATION

by

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THE SURVIVAL OF MESOPHILIC AND THERMOPHILIC BACTERIA SUBJECTED TO UV IRRADIATION

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Survival curves were determined for the mesophile, Bacillus licheniformis and the thermophile, Bacillus stearothermophilus after subjecting the cells to ultraviolet irradiation. Cells were grown at 37°C for the mesophile and at 55°C for the thermophile.

At low UV exposure times, the thermophile was more resistant to UV irradiation (had a better survival rate) than was the case for the mesophile. At higher exposure times, the situation was reversed, the mesophile having a greater survival rate than the thermophile.

The effect of changes in the temperature during irradiation was also determined. Thermophiles were greatly affected by changes in temperature while the mesophile appeared to be relatively insensitive to variations in temperature (except at a 50 second exposure time).

Varying the incubation temperature at which irradiated cells were allowed to grow led to significant changes in the survival curves of the thermophiles. The survival rate was greater when the cells were incubated at 63°C, as compared to their incubation at 55°C. However, changes in the incubation temperature had little or no effect on the survival curves of the mesophile.

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CHAPTER I

INTRODUCTION

It is now well established that UV light causes dimerization of pyrimidines in DNA and results in an increased rate of mutation. The relative efficiency of dimer formation is in the order of TT > CT > CC (1). Thymine dimers are present in native, unirradiated DNA in sufficient numbers to cause biological effects. However, the extent to which such effects are shown by an irradiated cell depends upon the ability of its enzymes to repair such lesions. The repair process utilizes DNA polymerase to excise damaged regions in DNA and to resynthesize new DNA as a replacement; the repair is completed by DNA ligase (2) which seals in the new fragment to the cell DNA.

Mesophiles are bacteria that grow optimally between 20°C and 45°C. Thermophiles,on the other hand, grow at much higher temperatures of about 45°C to 75°C (3,4). For this study, Bacillus licheniformis, a mesophile grown at 37°C, and Bacillus stearothermophilus, a thermophile grown at 55°C, were chosen as model systems. The effect of ultraviolet irradiation on cell survival and DNA repair capacity was evaluated by varying the UV intensity, the time and temperature of irradiation, and the temperature for bacterial growth subsequent to irradiation.

The bacterial culture to be irradiated was grown to the mid log phase. At this point, the growth fraction is high (usually 90-100%) and the culture is in its most reproducible form. The growth curves for these two organisms were determined earlier by Gupta and the mid log

phase was shown to correspond to an absorbance of 0.55 for the mesophile grown at 37°C and to an absorbance of 0.50 for the thermophile grown at 55°C (5). The absorbance of the culture, log (I_o/I), is proportional to the cell concentration. Absorbance measurement, therefore, represent a rapid and simple method for following the growth of bacterial cells. Absorbance was measured at 540 nm.

The number of cells, in a given aliquot, before and after irradiation,was determined by a plate count technique. This is based on the principle that, if the aliquot is suitably diluted and the cells spread out over a layer of agar in a petri dish, then each viable organism will grow into one colony. To this end, the original sample (culture grown to mid log phase) is usually diluted so that the number of colonies developing on the plate falls in the range of 30 to 300. Within this range the count is accurate and the possibility of interference of the growth of one organism with that of another is minimized (6). It is assumed, in using this technique, that the bacterial suspension is homogeneous and that no aggregates of cells are present.

This study was undertaken as part of the ongoing research in this laboratory into the nature of thermophily (7,8). The hypothesis that thermophiles differ from mesophiles in physical and chemical properties of important macromolecules has received a great deal of support thus far. This study was aimed at evaluating the applicability of this hypothesis as regards the stability of cellular DNA to UV irradiation.

CHAPTER II

MATERIALS AND METHODS

Media and Growth Conditions

Agar slants

Stock cultures of *B. licheniformis* (NRS 243) and *B. stearothermophilus* 10 were maintained on agar slants. The slants consisted of 1% Trypticase (BBL), 0.2% Yeast Extract (Difco), and 2% Bacto agar (Difco). The stock cultures were grown at 37°C for the mesophile and at 55°C for the thermophile and then stored in a refrigerator (2-4°C). Ordinary slants had the same composition as the stock cultures and were prepared from them by inoculating with a sterile tranfer loop (6); they were grown at 37°C and 55°C, respectively.

Liquid medium

Cells of *B. licheniformis* and *B. stearothermophilus* were grown at 37°C and 55°C, respectively, in flasks containing liquid medium. The medium consisted of 1% Trypticase (BBL) and 0.2% Yeast Extract (Difco). Flasks containing the bacterial culture were incubated in an incubator shaker (New Brunswick Scientific, Model G-25). Sterile Liquid medium was used both for growing bacteria and for diluting aliquots of cell culture for plate counts.

Growth of Cells

The overnight growth from one agar slant for each organism was washed off with 10 mL of sterile liquid medium. The wash solution was poured into 100 mL of fresh sterile liquid medium and the cell culture was then incubated at 37°C for B. lichenformis and at 55°C for B. stearothermophilus in the incubator shaker. After about 2½ hours to 3 hours of incubation, 5.0 mL of the culture was transferred into another 100 mL of fresh liquid medium and allowed to grow again for 3 to 4 hours at 37°C and 55°C, respectively. At this point, the culture had reached an absorbance of about 0.5 at 540 nm, which is approximately the mid log phase for both the mesophile and the thermophile (5). The cells were not allowed to exceed the late log phase at any time. The late log phase corresponds to an absorbance of 0.7 at 540 nm for the mesophile and an absorbance of 0.65 for the thermophile (5).

Dilution of the Cells

Liquid cultures of B. lichenformis and B. stearothermophilus that were grown to an absorbance of about 0.5 have a cell count of approximately 1×10^8 cells/mL (5). These cultures had to be diluted prior to plating so that the number of colonies developing on the plate would fall within the range of 30 to 300. To this end, the original sample ($A_{540} = 0.5$) was diluted a million fold with liquid medium (1:1,000,000 dilution). The diluted sample of B. lichenformis was kept at room temperature, but the diluted sample of B. stearothermophilus was maintained at 37°C because thermophilic cells tend to become unviable if stored at room temperature.

UV Irradiation and Cell Plating

Basic Procedure

A sample of 0.5 mL of an appropriately diluted bacterial culture was transferred to a sterile disposable plastic petri dish (100 x 15 mm) and exposed to shortwave (254 nm) ultraviolet light (Mineralight Model R-51, 115 volts, 50-60 cycle, 0.6 ampere). The UV lamp was switched on 15 minutes prior to irradiation of the sample in order to stabilize the emission of the lamp. The ultraviolet intensity at the position of the petri dish was determined by means of a Blak-Ray, shortwave ultraviolet meter (Model J225)(254 nm) (9). The cell suspension was exposed to the UV light for a given time period while agitating the suspension gently. Accurate timing was achieved by removing the petri dish cover under the UV light for the required time and then quickly replacing it. No UV light passed through the plastic cover.

The samples were irradiated for 10, 20, 30, 40, and 50 seconds. The control,or the zero time, was the unirradiated sample. After irradiation, 15 to 20 mL of sterile agar slant medium (2% Yeast Extract, 1% Trypticase, and 2% Bacto agar) was poured into each plate. The plate was gently rotated for thorough distribution of the culture throughout the agar.

The agar medium begins to solidify at 40°C. In order for it to be in the liquid state, prior to pouring, it was maintained at 55°C, in a holding oven. This agar medium (at 55°C) was used directly for pouring the thermophile plates but it was first cooled to approximately 45°C for the mesophile plates (the agar was allowed to cool for 10-12 minutes

at room temperature prior to pouring).

After mixing the cells with the agar medium, the plates were incubated for 24 hours in an incubator. Incubation was at 37°C for B. lichenformis and at 55°C for B. stearothermophilus.

Under these conditions, each cell gives rise to a simple colony. Hence, a colony count of the plate yields the number of viable microbial cells in the culture sample. This is known as a viable cell count. The colonies were counted with the help of a simple magnifying colony counter. All of the experiments were performed in triplicate and the cell counts were within 10% of the mean. Additionally, for the figures, all the data were normalized to an initial cell count (zero time, unirradiated sample) of 316 colonies per plate (log [S] = 2.5)

Effect of Ultraviolet Intensity on Cell Survival

For this experiment, 0.5 mL of diluted cell suspension of either B. licheniformis or B. stearothermophilus were irradiated at three different ultraviolet intensitites: 4.4 J/sec/m^2, 6.0 J/sec/m^2 and 8.0 J/sec/m^2. Irradiation was at room temperature and for each intensity the exposure time was varied from zero to 50 seconds, using increments of 10 seconds.

Effect of Irradiation Temperature on Cell Survival

For this experiment, 0.5 mL of diluted cell suspension of either B. licheniformis or B. stearothermophilus were irradiated at three different temperatures, using a fixed ultraviolet intensity of 6.0 J/sec/m^2 and varying the exposure time from zero to 50 seconds in 10 second intervals.

For B. licheniformis, the irradiation was carried out at room temperature, at 30°C, and at 43-45°C. For B. stearothermophilus, the irradiation was carried out at room temperature, at 44-48°C and at 60-63°C. The organisms are known to be viable in these temperature ranges.

To achieve the different irradiation temperatures, the petri dish was placed in a regulated hot plate; for the 60-63°C experiment, glass petri dishes were used. The temperature was measured by immersing a thermometer in a petri dish filled with H_2O.

The cell suspension was preheated on the hot plate for about 20 seconds prior to irradiation. After irradiation, the plates were incubated as usual for 24 hours, at 37°C for B. licheniformis and at 55°C for B. stearothermophilus.

Effect of Temperature on the Growth of Cells

For this experiment, 0.5 mL of diluted bacterial suspension of either B. licheniformis or B. stearothermophilus were irradiated at an ultraviolet intensity of 6.0 J/sec/m^2. Irradiation was at room temperature followed by plating of the cells. Subsequent to plating, however, the petri dishes were incubated at various temperatures. For B. licheniformis, the incubation temperatures were 30°C, 37°C, and 45°C; for B. stearothermophilus, the incubation temperatures were 45°C, 55°C, and 63°C. The plates were incubated for 24 hours as usual.

Effect of Multiple Irradiation on Cell Survival

For this experiment, cells of B. licheniformis that survived 50 seconds irradiation at room temperature using an ultraviolet intensity

of 4.4 J/sec/m^2, were allowed to grow and were then irradiated once more at the same UV intensity and at the same temperature.

The colonies appearing after the first irradiation were scraped off from one petri dish and used as an inoculum for one fresh agar slant. The latter was grown twice at 37°C for 12 hours (one transfer) and then transferred to liquid medium and allowed to grow in the usual manner. The culture thus obtained was diluted as above and was then irradiated at room temperature at 4.4 J/sec/m^2 for 0, 10, 30, and 50 seconds. After pouring, the plates were incubated at 37°C for 24 hours.

CHAPTER III

RESULTS AND DISCUSSION

Viable Cell Count

Dilutions were tried in order to determine the initial cell concentration of the culture at an absorbance of 0.5 at 540 nm. A $1:10^6$ dilution yielded a cell count of approximately 300 cells/mL. On this basis the initial cell concentration was calculated to be 3×10^8 cells/mL. In all of the experiement 0.5 mL of $1:10^6$ diluted culture was plated in order to obtain approximately 150 colonies per plate.

Effect of Ultraviolet Intensity on Cell Survival

The effect of variable ultraviolet intensities on the survival of B. licheniformis and B. stearothermophilus cells is shown in Figure 1 and Figure 2, respectively. In these figures, the logarithm of the number of colonies (viable cell count) is plotted against the time (seconds) of UV exposure. Such a plot is known as a survival curve. In these figures and tables, and in all subsequent figures and tables, [S] denotes the number of colonies per plate. This is an average of three separate deteminations, since all of the experiments were done in triplicate.

It can be seen from Figures 1 and 2 and Tables 1 and 2 that, at low exposure times (10 and 20 seconds), both the mesophile and the thermophile survival rate decreased as the UV intensity was increased. Moreover,

Table 1

Effect of UV Intensity on the Survival of B. Licheniformis Cells

	Ultraviolet Intensity					
	4.4 J/sec/m^2		6.0 J/sec/m^2		8.0 J/sec/m^2	
UV Exposure (seconds)	Number of Colonies [S]	Log[S]	Number of Colonies [S]	Log[S]	Number of Colonies [S]	Log[S]
zero	188	2.27	211	2.32	224	2.35
10	87	1.94	57	1.76	47	1.67
20	19	1.28	25	1.40	27	1.43
30	26	1.41	25	1.40	23	1.36
40	34	1.53	25	1.40	16	1.20
50	14	1.15	15	1.18	16	1.20

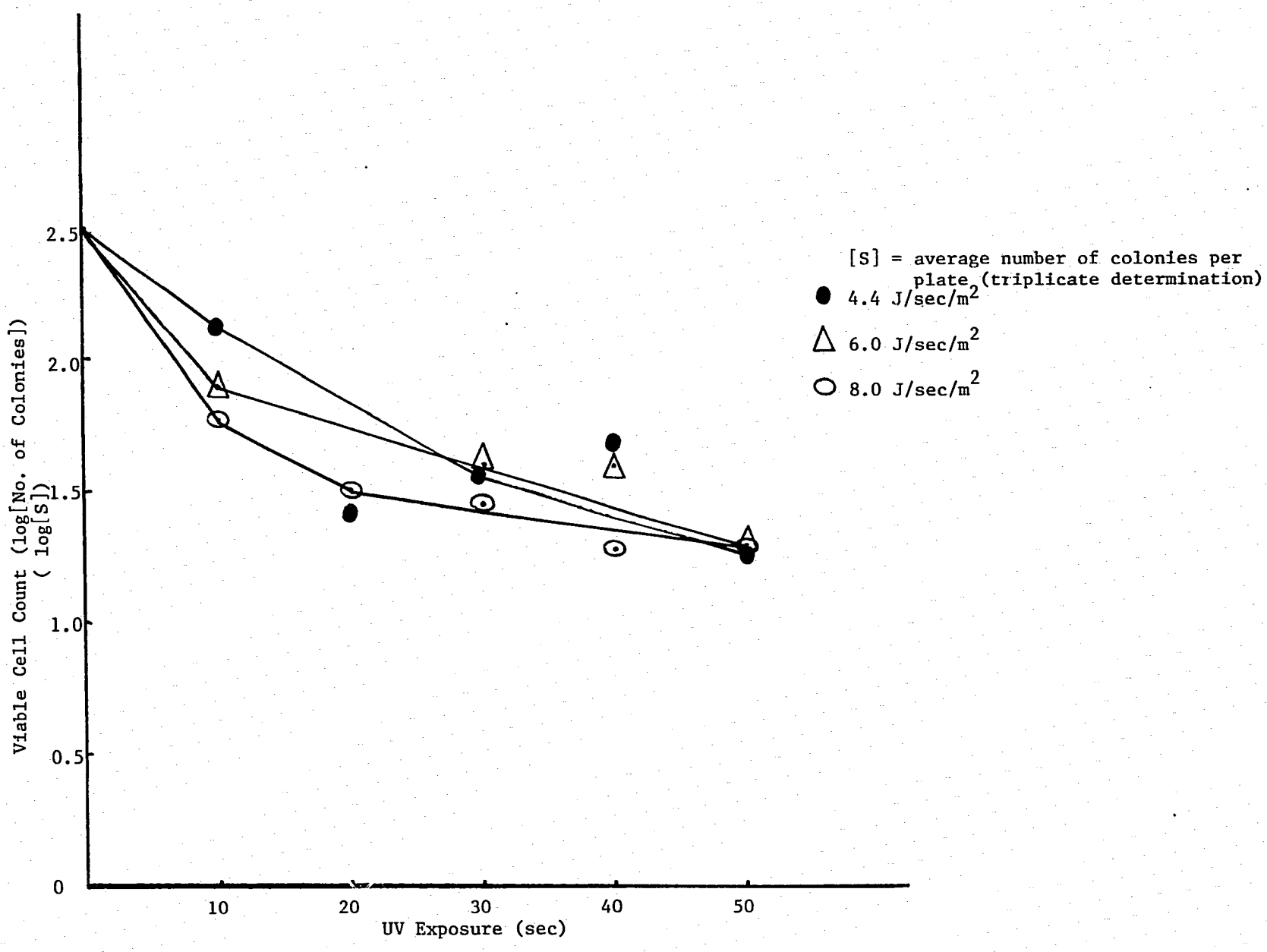

Figure 1. Survival Curves of B. Licheniformis Cells, Irradiated at Room Temperatures and Variable Ultraviolet Intensities.

Table 2

Effect of UV Intensity on the Survival of B. Stearothermophilus Cells

	Ultraviolet Intensity					
	4.4 J/sec/m^2		6.0 J/sec/m^2		8.0 J/sec/m^2	
UV Exposure (seconds)	Number of Colonies [S]	Log[S]	Number of Colonies [S]	Log[S]	Number of Colonies [S]	Log[S]
zero	103	2.01	134	2.13	169	2.23
10	82	1.91	85	1.93	93	1.97
20	54	1.73	38	1.58	32	1.51
30	44	1.64	6	0.78	21	1.32
40	15	1.18	2	0.30	14	1.15
50	7	0.84	2	0.30	9	0.95

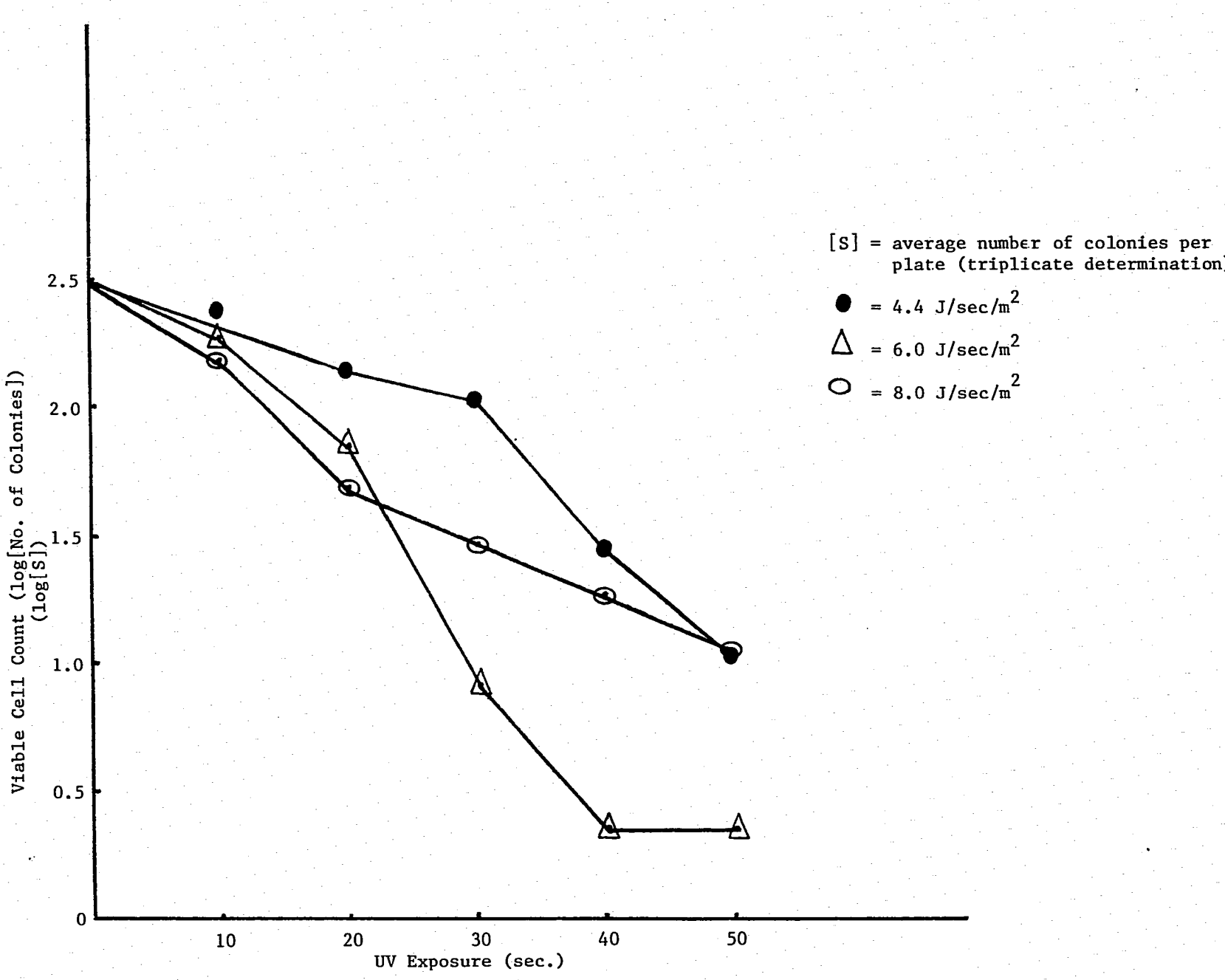

Figure 2. Survival Curves of B. Stearothemophilus cells, Irradiated at Room Temperatures and Variable Ultraviolet Intensities.

the mesophile was more sensitive to irradiation; the number of surviving colonies decreased faster than was the case for the thermophile.

At higher exposure times (30, 35, and 50 seconds), the survival curve for the mesophile was essentially independent of the UV intensity. The survival curve for the thermophile, on the other hand, showed a pronounced decrease; the number of surviving cells decreased as the UV intensity was increased.

To further highlight these differences, the survival curves for both organisms, at a given UV intensity, have been plotted on one graph. These curves are shown in Figures 3, 4, and 5.

It is again apparent that, at low exposure times, the thermophile is more resistant to UV irradiation (has a better survival rate) than is the case for the mesophile. At higher exposure times, the situation is reversed, the mesophile having a greater survival rate than the thermophile.

Lethal UV Dose

The data shown in Tables 1 and 2 can be recalculated so as to indicate the approximate dose of UV (in Joules) required to kill one cell.

TO do this we first calculate the area of the petri dish

$$\pi r^2 = (\pi 5^2) = 78.5 \text{ cm}^2 = 7.85 \times 10^{-3} \text{ m}^2$$

The total UV dose per petri dish is then given by

$$(\text{UV intensity})(7.85 \times 10^{-3} \text{ m}^2)(\text{exposure time}).$$

As an example, for a UV intensity of 4.4 J/sec/m^2 and a 20 second exposure, the lethal UV dose is

$$(4.4 \text{ J/sec/m}^2)(7.85 \times 10^{-3} \text{ m}^2)(20 \text{ sec}) = 0.68 \text{ J}$$

We next obtain the number of cells (colonies) killed by this UV dose by

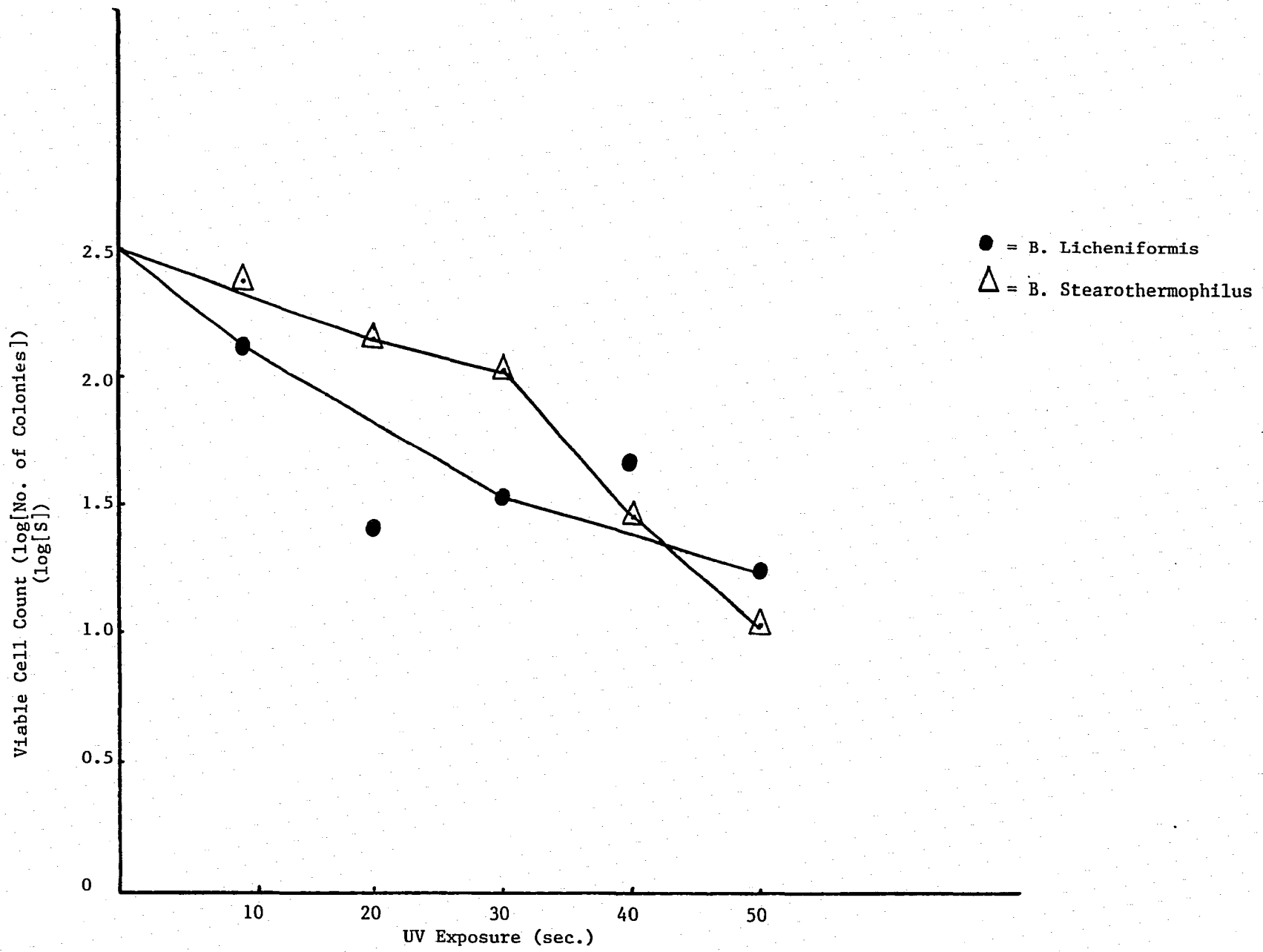

Figure 3. Survival Curves of B. Licheniformis and B. Stearothermophilus Cells, Irradiated at Room Temperature and an Ultraviolet Intensity of 4.4 J/sec/m^2.

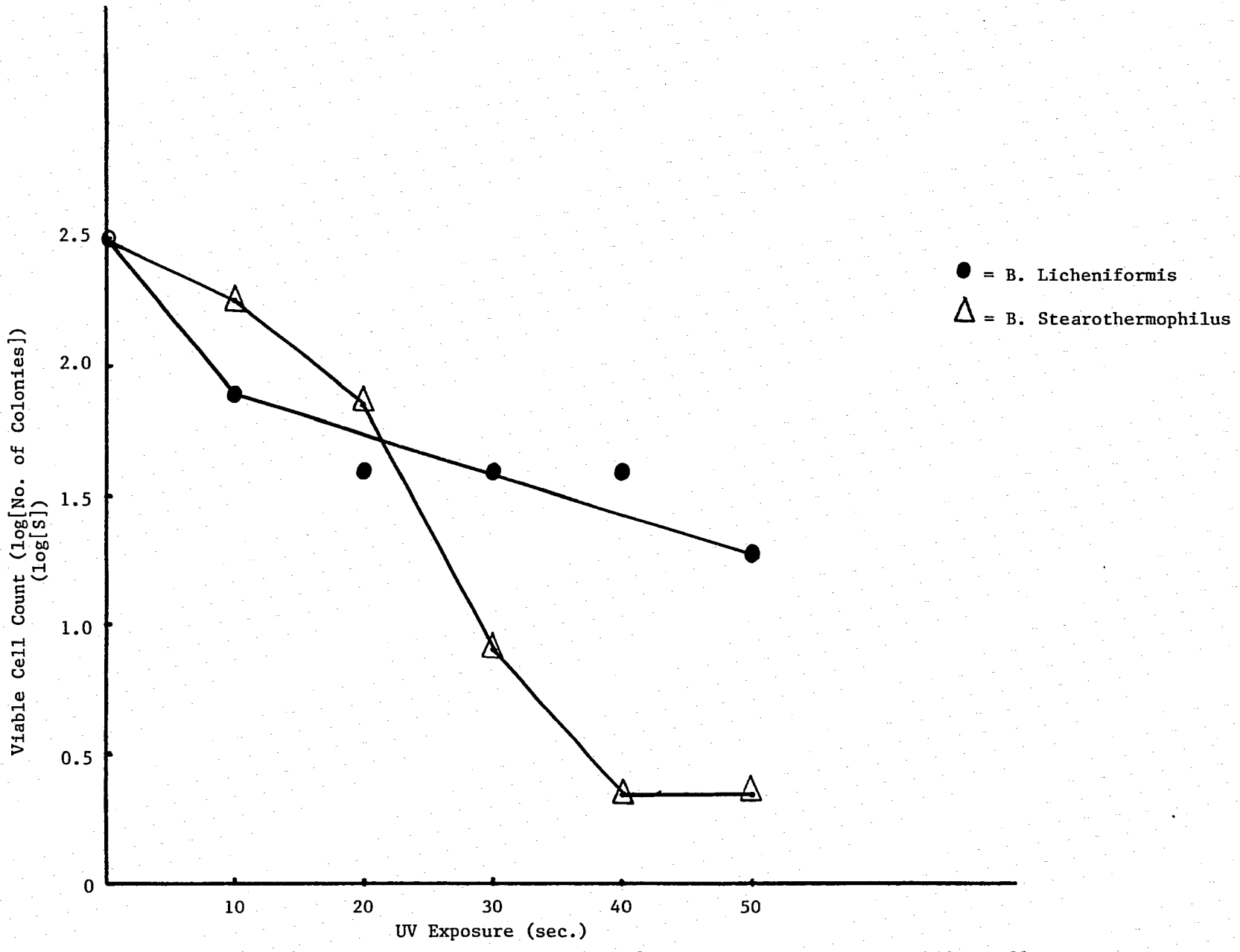

Figure 4. Survival Curves of B. Licheniformis and B. Stearothermophilus cells, Irradiated at Room Temperature and at an UV Intensity of 6.0 J/sec/m^2

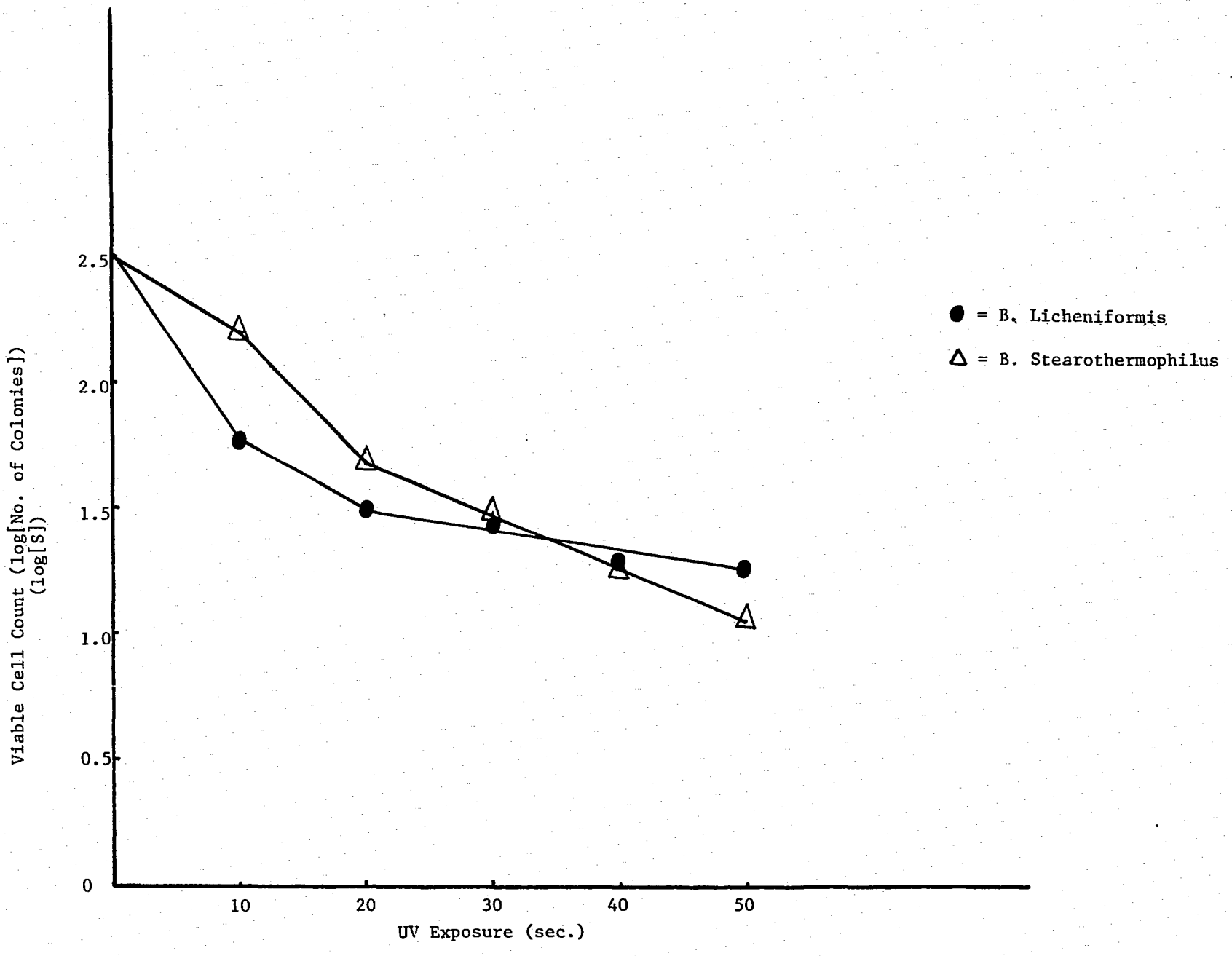

Figure 5. Survival Curves of B. Licheniformis and B. Stearothermophilus cells Irradiated at Room Temperature and at an UV Intensity of 8.0 J/sec/m^2

subtracting the number of colonies counted after 20 seconds exposure from the number counted at zero time (no exposure). Dividing this number of cells killed, by the total UV dose used gives the approximate UV dose required to kill one cell colony. These calculated values are shown in Tables 3 and 4.

It can be seen from Tables 3 and 4 that the mesophile required much less UV doses for killing of a cell than thethermophile,especially at low exposure times. Furthermore, this dose increased greatly as the exposure time was increased. For the thermophile, on the other hand, the dose/cell was either essentially constant, regardless of exposure time, or increased to a smaller extent than for the mesophile.

These data indicate an overall greater sensitivity ofthe thermophile to UV irradiation.

Effect of Irradiation Temperature on Cell Survival

The effects of varying the temperature during the irradiation on the survival of B. licheniformis and B. stearothermophilus are shown in Figures 6 and 7 and Tables 5 and 6. There was some variation in the final temperature of the plate depending on the length of time that a petri dish was irradiated and kept on the hot plate. Therefore, the temperature in Tables 5 and 6 are listed in the following manner:

For B. licheniformis: room temperature as room temp. (no uncertainty), 30°C as 30°C (no uncertainty because walk-in incubator was used), and 40-45°C as $> 37^{\circ}C$.

For B. stearothermophilus: room temperature as room temp. (no uncertainty), 45-48°C as $< 55^{\circ}C$, and 60-63°C as $> 55^{\circ}C$.

Table 3

Approximate UV Dosage Required to Kill One Cell Colony of B. Licheniformis as a Function of UV Dose

UV Exposure (seconds)	Ultraviolet Intensity 4.4 J/sec/m^2 Cells Killed	(x 10^3) Dose/cell	6.0 J/sec/m^2 Cells Killed	(x 10^3) Dose/cell	8.0 J/sec/m^2 Cells Killed	(x 10^3) Dose/Cell
zero	0	---	0	---	0	---
10	101	3.36	154	3.06	177	3.54
20	169	4.09	186	5.06	197	6.37
30	162	6.40	186	7.60	201	9.37
40	154	8.97	186	10.13	208	12.07
50	174	9.92	196	12.01	208	15.10

Table 4

Approximate UV Dosage Required to Kill One Cell Colony of B. Stearothermophilus as a Function of UV Dose

	Ultraviolet Intensity					
	4.4 J/sec/m^2		6.0 J/sec/m^2		8.0 J/sec/m^2	
UV Exposure (seconds)	Cells Killed	(x 10^3) Dose/cell	Cells Killed	(x 10^3) Dose/cell	Cells Killed	(x 10^3) Dose/Cell
zero	0	---	0	---	0	---
10	21	16.45	49	9.61	76	8.26
20	49	14.10	96	9.81	137	9.16
30	59	17.56	128	11.04	148	12.70
40	88	15.70	132	14.27	155	16.21
50	96	17.99	132	17.84	160	19.62

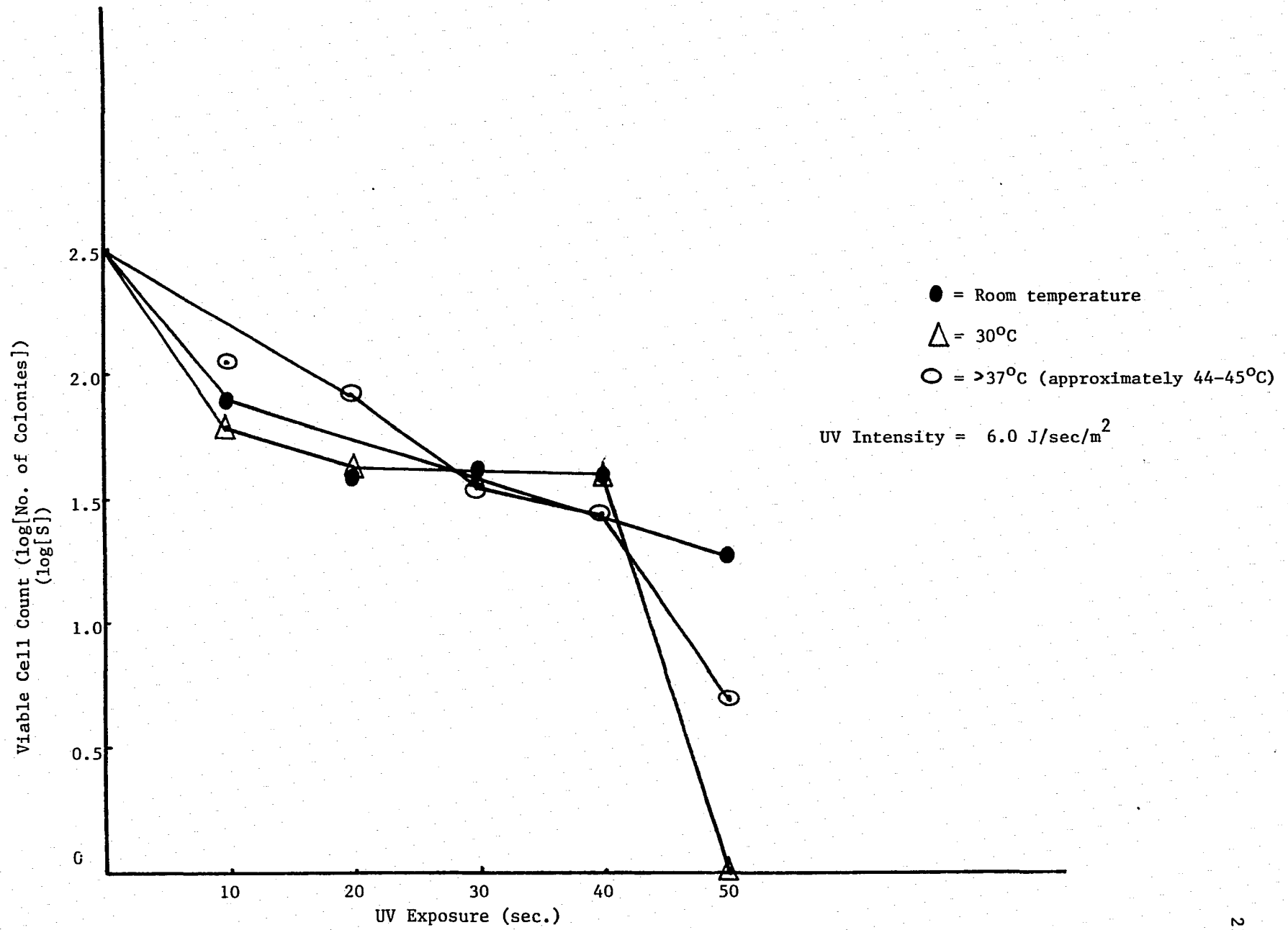

Figure 6. Survival Curves of B. Licheniformis cells, irradiated at a Fixed UV Intensity and at Varying Temperatures.

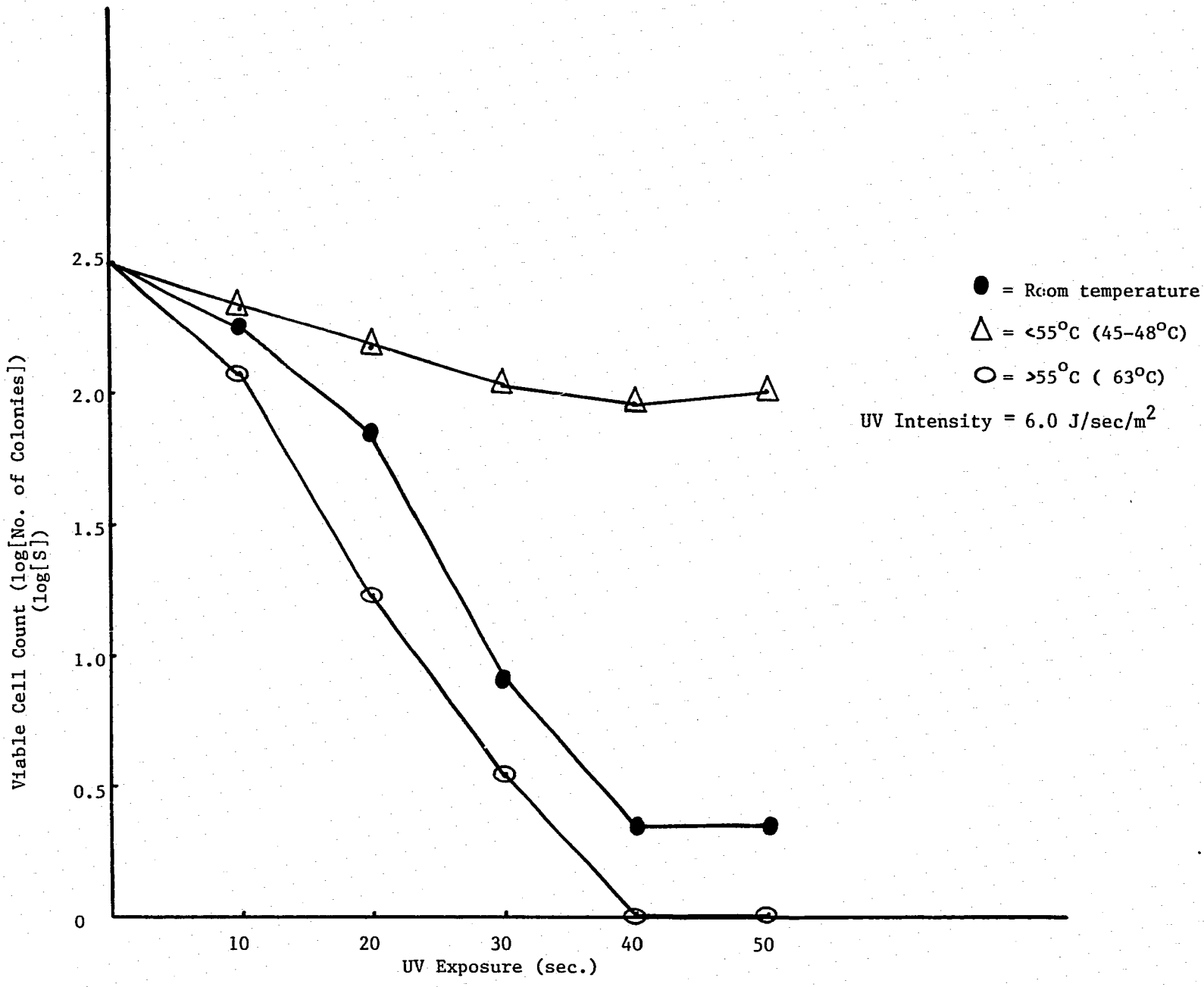

Figure 7. Survival Curves of B. Stearothermophilus Cells, Irradiated at a Fixed UV Intensity and at Varying Temperatures

Table 5
Effect of Irradiation Temperature on the Survival of
B. Licheniformis cells

UV Exposure (Seconds)	Irradiation Temperature: Room Temperature Number of Colonies [S]	Log[S]	30°C Number of Colonies [S]	Log[S]	>37°C Number of Colonies [S]	Log[S]
zero	211	2.32	158	2.20	149	2.17
10	57	1.76	37	1.57	61	1.78
20	25	1.40	27	1.43	46	1.66
30	25	1.40	24	1.38	23	1.36
40	25	1.40	25	1.40	18	1.25
50	15	1.18	1	0.00	4	0.60

Table 6
Effect of Irradiation Temperature on the Survival of
B. Stearothermophilus Cells

UV Exposure (seconds)	Irradiation Temperature: Room Temperature Number of Colonies [S]	Log[S]	<55°C Number of Colonies [S]	Log[S]	>55°C Number of Colonies [S]	Log[S]
zero	134	2.13	150	2.18	159	2.20
10	85	1.93	106	2.02	67	1.83
20	38	1.58	78	1.89	12	1.08
30	6	0.78	59	1.77	3	0.48
40	2	0.30	38	1.58	0	0.00
50	2	0.30	41	1.61	0	0.00

It is apparent from Figures 6 and 7 that a change in the temperature of irradiation has a significant effect on the survival curves. But the change is complex and not readily interpreted. For each organism, one curve falls above that of room temperature, and one falls below, despite the fact that both curves represent temperatures above room temperature. Additionally, greatest sensitivity (lowest survival) appears to be at 30°C for the mesophile and at 55°C for the thermophile; precisely the temperatures at or close to the optimum growth temperature for these organisms. Lastly, the mesophile appeared to be relatively insensitive to variations in temperature (except at 50 seconds exposure time) while the survival curves of the thermophile were greatly affected by changes, of temperature. Offhand, the opposite effect might have been expected.

Effect of Temperature on the Growth of Irradiated Cells

Varying the incubation temperature at which irradiated cells are allowed to grow represents one way of assessing the UV repair mechanisms in the organism.

As far as the mesophile was concerned such changes, in temperature, had little, if any, effect on the survival curves (See Table 7, Figure 8). However, B. Stearothermophilus, when incubated at 63°C, showed a significant increase in the survival of cells compared to that at 55°C (See Table 8, Figure 9). (B. Stearothermophilus, at an incubation temperature of 45°C, did not show any growth on the agar plate after 24 hours. It appears that, at this temperature a longer incubation period is required.) One might tentatively conclude that, in B. Stearothermophilus, the lesions caused by UV irradiation are more effectively repaired at 63°C than at 55°C.

Table 7
Effect of Incubation Temperature on the Growth of Irradiated
B. Licheniformis Cells
(UV Intensity 6.0 J/sec/m^2)

UV Exposure (seconds)	Incubation Temperature 30°C Number of Colonies [S]	30°C Log[S]	37°C Number of Colonies [S]	37°C Log[S]	45°C Number of Colonies [S]	45°C Log[S]
zero	183	2.36	211	2.32	106	2.0
10	53	1.72	57	1.76	37	1.57
20	22	1.34	25	1.40	21	1.32
30	8	0.90	25	1.40	7	0.84
45	16	1.20	25	1.40	9	0.95
50	10	1.00	15	1.18	5	0.70

Table 8
Effect of Incubation Temperature on the Growth of UV Irradiated
B. Stearothermophilus Cells
(UV Intensity 6.0 J/sec/m^2)

UV Exposure (seconds)	Incubation Temperatures 45°C* Number of Colonies [S]	45°C* Log[S]	55°C Number of Colonies [S]	55°C Log[S]	63°C Number of Colonies [S]	63°C Log[S]
zero			134	2.13	170	2.23
10			85	1.93	116	2.06
20			38	1.58	72	1.86
30			6	0.78	57	1.75
45			2	0.30	29	1.46
50			2	0.30	14	1.15

*Colonies did not appear after 24 hours of incubation.

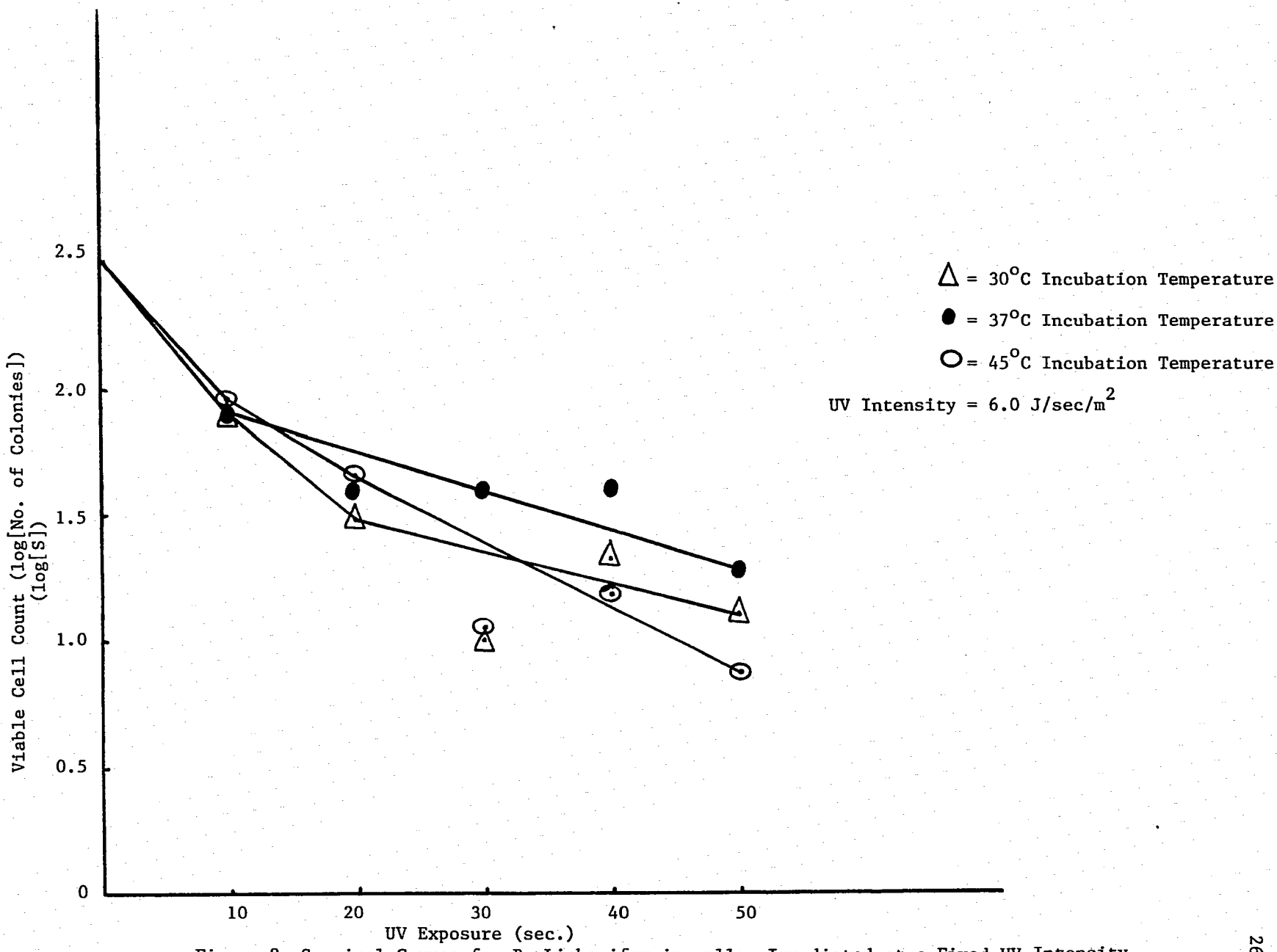

Figure 8. Survival Curves for B. Licheniformis cells, Irradiated at a Fixed UV Intensity at Room Temperature and then Incubated at Various Temperatures.

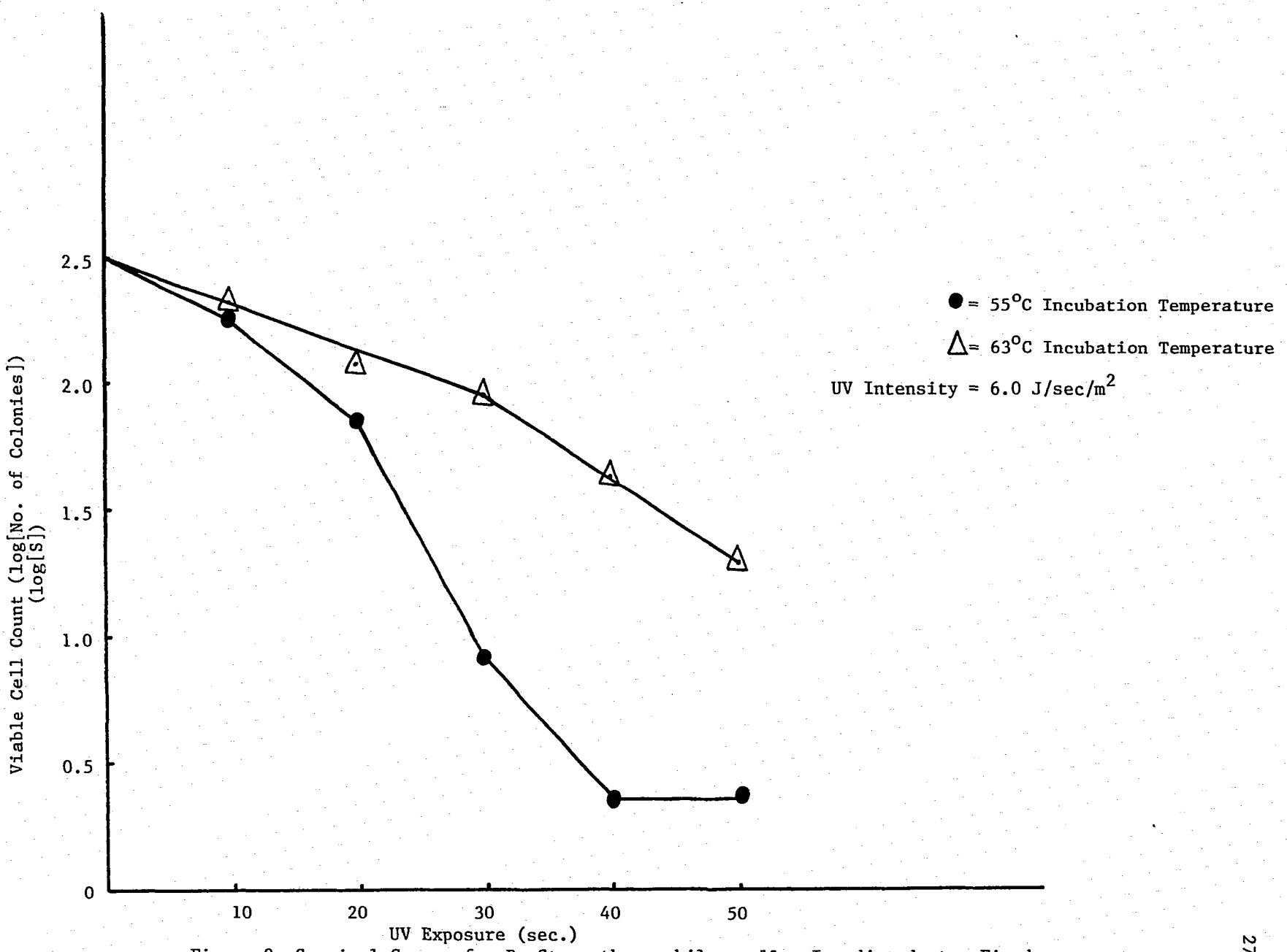

Figure 9. Survival Curves for B. Stearothermophilus cells, Irradiated at a Fixed UV Intensity at Room Temperature and then Incubated at Various Temperatures

Effect of Multiple Irradiation on Cell Survival

The purpose of this experiment (See Chapter II) was to investigate whether cells that survived 50 seconds of irradiation represented cells that were inherently resistant to that dose of UV irradiation or whether they represented cells that survived as a result of a well-functioning repair system. As can be seen from Figure 10 (Table 9), there was no significant difference between the survival curve of the

Table 9

Effect of UV Intensity (4/4 J/sec/m^2) on the Survival of Recultivated B. Licheniformis that survived 50 Seconds of UV Radiation

UV Exposure (seconds)	UV Intensity (4.4 J/sec/m^2) Number of Colonies [S]	Log[S]
zero	137	2.14
10	62	1.79
30	12	1.08
50	12	1.08

initial culture and the survival curve of a culture derived from such "UV resistant" cells. This indicated that cell survival to UV radiation is not so much due to inherent stability to UV but rather due to an efficient repair mechanism.

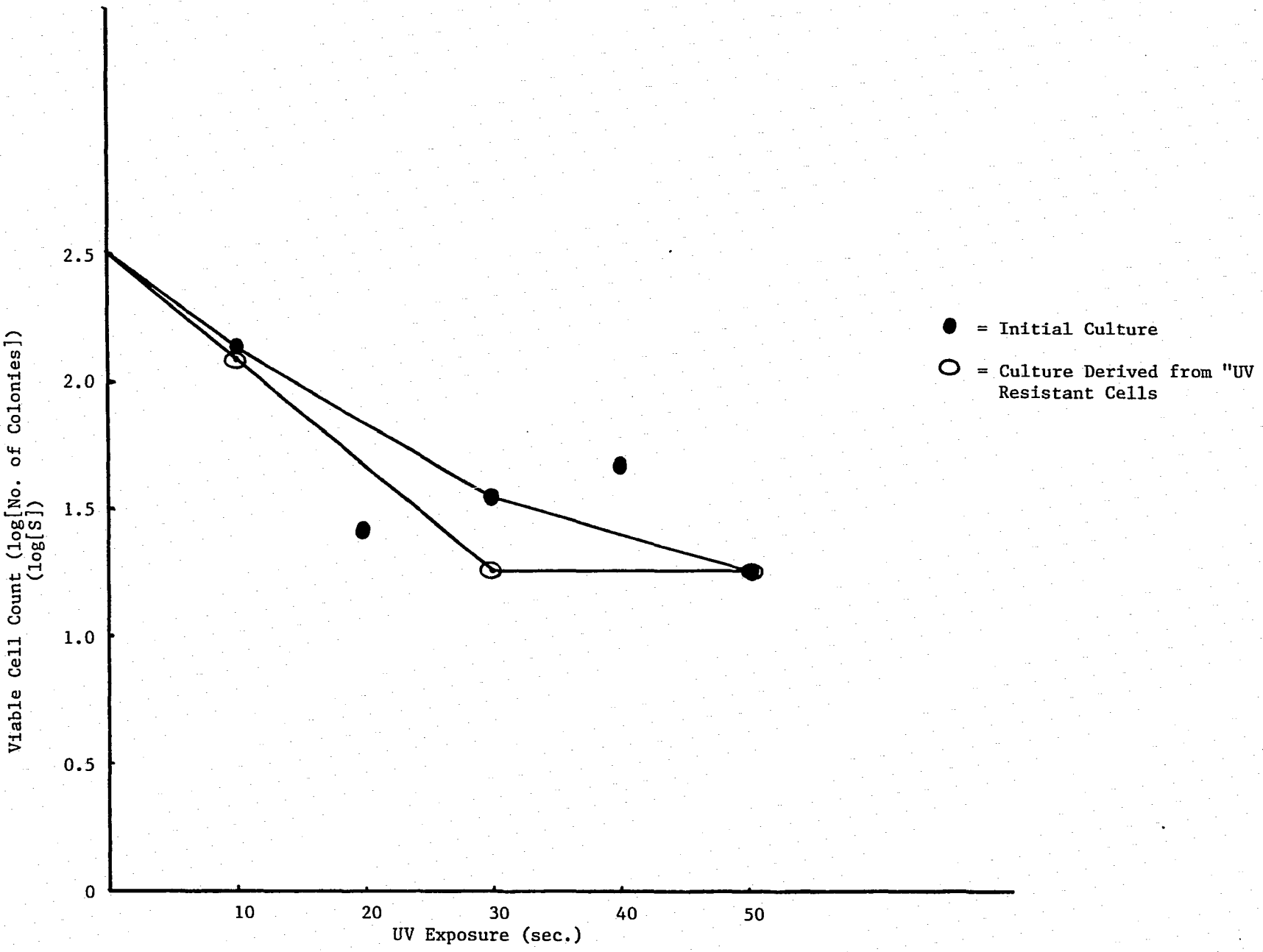

Figure 10. Survival Curves of B. Licheniformis Irradiated at Room Temperature and at an UV Intensity of 4.4 J/sec/m^2.

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