APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1980, p. 695-701
0099-2240/80/04-0695/07$02.00/0
Vol. 39, No. 4

Resistance and Recovery Studies on Ultraviolet-Irradiated Spores of *Bacillus pumilus*

ROBERT L. ABSHIRE,* BEVERLY BAIN, AND THELMA WILLIAMS

Product Services Microbiology, Alcon Laboratories, Inc., Fort Worth, Texas 76101

A spore suspension model and a procedure for recovering ultraviolet (UV)-irradiated spores of *Bacillus pumilus* were investigated. A most-probable-number tube dilution method using double-strength Trypticase soy broth was found to be superior to the agar plate method for recovering optimal numbers of spores irradiated with sublethal doses of UV energy. Aqueous suspensions of *B. pumilus* survived UV doses up to 108,000 ergs/mm^2 as determined by a most-probable-number recovery and estimation procedure. Resistance and stability data were consistent and reproducible, indicating the dependability of this method for recovering UV-damaged spores. The procedures used to collect information concerning resistance characteristics for two strains of *B. pumilus* are discussed.

Spores of *Bacillus pumilus* E601 (ATCC 27142) are used as biological indicators to certify and monitor the efficacy of commercial radiosterilization cycles. The resistance of this organism to gamma radiation has been evaluated (2, 15; I. T. Zuk, A. N. Parisi, and P. M. Borick, Annu. Meet. Am. Soc. Microbiol., 1972), and it has been shown to be one of the most resistant microorganisms to this sterilizing process (3, 19).

Many articles involving the effects of ultraviolet (UV) radiation on spores have been published. However, to our knowledge, information of this nature concerning *B. pumilus* is unavailable. The primary objectives of this study were to determine the resistance of spores from two strains of *B. pumilus* to UV, and to devise and evaluate an optimal recovery system for spores of *B. pumilus* subjected to sublethal doses of UV radiation.

MATERIALS AND METHODS

Bacterial strains. *B. pumilus* E601 was cultured from a spore strip obtained from Ethicon, Inc. (Somerville, N.J.). Strain BMT-18 was isolated (Alcon Laboratories, Inc., Fort Worth, Tex.), submitted to R. Gordon, Rutgers University, and identified as *B. pumilus*.

Preparation of spore suspensions. Stock spore suspensions of *B. pumilus* E601 and BMT-18 were prepared by inoculating the surface of AK agar no. 2 (BBL Microbiology Systems, Cockeysville, Md.) contained in Roux bottles (800-ml capacity, 250 ml of agar) with 3 ml of cells from a 24-h Trypticase soy broth (TSB; BBL) culture. The bottles were incubated at 32 to 35°C for 7 days, by which time sporulation had reached 90 to 95%. Spores were harvested from the agar surface with sterile deionized water, collected by centrifugation (1,800 × *g*, 15 min), and washed twice with 15 ml of sterile deionized water. After resuspension in 15 ml of sterile deionized water, the spore pastes were heated in a water bath at 80°C for 20 min to destroy the vegetative cells. The spores were again collected by centrifugation and resuspended in 25 ml of sterile deionized water, constituting spore concentrates. Viable spore counts were determined, and working suspensions of 10^7 to 10^9 spores per ml were prepared from each concentrate. The working suspensions were diluted further to the desired number of spores for each radiation study.

Spore suspension model. Spore suspensions for each viability and resistance study were prepared in 1,000-ml quantities and stored in 2-liter glass flasks. Immediately before each study, the spore suspensions were mixed for 30 min with the aid of a magnetic stirrer and bar, and were dispensed in 2-ml portions into 15-ml S/R Natural Drop-Tainers (NDT, Alathon 20 low-density polyethylene, Wheaton Industries, Millville, N.J.). Each NDT was covered and sealed with a polyethylene flat fitment (15 mm, Alathon 20 low-density polyethylene, Brown Research, Burbank, Calif.). Based on microbiological and physical studies, these particular NDTs were determined to allow transmittance of approximately 45% of the UV light absorbed, and the flat fitments permitted essentially 100% transmittance (B. A. Schlech, unpublished data).

Exposure to UV radiation. Duplicate sets of spore suspensions prepared in NDTs were exposed to incremental doses of UV radiation. The source of UV radiation was a Westinghouse low-pressure mercury vapor shortwave UV lamp (Sterilamp, model 782L 30, Westinghouse Electric Corp., Pittsburgh, Pa.). The lamp was installed in a cabinet (about 105 by 30 by 45 cm) with an adjustable shelf which could be positioned at a desired distance from the UV lamp. In each radiation study, 2-ml spore suspensions contained in NDTs were exposed to a constant intensity of 1,000 μW/cm^2 (distance from the bulb to spore suspension, about 10 cm) for various lengths of time at ambient temperature and relative humidity. The UV intensity was determined for each experiment with a Blak-Ray UV intensity meter (model J-225, Ultraviolet Prod-

ucts, San Gabriel, Calif.) which measures wavelengths of light energy between 230 and 270 nm with peak sensitivity at 254 nm. This instrument was calibrated and certified by Ultraviolet Products with traceability to a National Bureau of Standards transfer meter (Eppley Labs, Newport, R.I.).

Survival curves were obtained by plotting the number of survivors of the initial spore populations against radiation doses (i.e., intensity multiplied by time in seconds). The least-squares method of analysis was used to calculate regression lines for each set of data, and D_{UV} (decimal reduction time) values were determined from the linear portions of the survival curves.

The following terms are adopted for use in this paper: (i) UV intensity is expressed as microwatts per square centimeter of UV energy; (ii) UV dose is defined as the radiation energy delivered to an NDT containing a spore suspension and is expressed in ergs per square millimeter; and (iii) D_{UV} is defined as the exposure time in minutes at a constant UV intensity of 1,000 $\mu W/cm^2$ required to reduce the initial spore population by 1 logarithm (90%).

Recovery medium studies. Pour plate methods (24 ml of agar in 100-by-15-mm petri plates) using Trypticase soy agar (TSA; BBL), brain heart infusion agar (BHIA; Difco Laboratories, Detroit, Mich.), Mueller-Hinton agar (MHA; BBL), Todd-Hewitt agar (THA; BBL), and nutrient agar (NA; BBL) were used to determine spore counts. Plates were counted after incubation at 32 to 35°C for 48 h.

For the purpose of comparison, TSB (BBL), brain heart infusion broth (BHIB; Difco), Mueller-Hinton broth (MHB; BBL), Todd-Hewitt broth (THB; BBL), and Columbia broth (CB; BBL) were prepared in double-strength (2×) concentrations and dispensed in 10-ml quantities in test tubes (18 by 150 mm) with plastic caps. TSB and CB were also prepared in single-strength concentrations and tested. These media were inoculated with appropriate 10-fold serial dilutions of unexposed or UV-irradiated spores. Transfer of irradiated spores into outgrowth media was made within 30 min after exposure. All tubes were incubated at 32 to 35°C for 5 days. A three-tube most-probable-number (MPN) procedure was used to estimate the number of spores recovered. The MPN indices for the number of spores recovered were computed from the three-tube statistical table (1).

Characterization of strains. Parent and surviving spores were identified morphologically and biochemically by the tests described by Gordon et al. (12).

RESULTS

Plate count studies. Plate count determinations for the five agar media tested are given in Table 1. Except for lower counts obtained on NA, the numbers of unexposed spores recovered with the other agar media were comparable.

Sensitivity of the MPN procedure. Table 2 contains the results accumulated from studies in which MPN and plate counts using TSB and TSA, respectively, were compared. The numbers of unexposed, viable spores recovered by the two procedures were in close agreement, demonstrating the MPN method to be as dependable and sensitive as the agar plate method.

Comparison of MPN broth media. Four different broth media were prepared in 2× concentrations and used in an MPN procedure to recover spores of *B. pumilus* E601 and BMT-18 which had not been irradiated with UV (Table 2). TSB, MHB, and THB gave similar and somewhat higher MPN estimates than BHIB. Based on these results, TSB was chosen to be used in additional studies.

Comparisons were made between single- and double-strength concentrations of both TSB and CB (Table 3). Of the four formulations tested, 2× TSB proved to be the most effective medium for recovering *B. pumilus* spores irradiated with incremental doses of UV. The calculated D_{UV} value for 2× TSB was 3.9 min, whereas the decimal reduction time for 2× CB was determined by the least-squares method to be 2.1 min. A 90% reduction value of 2.9 min was obtained with single-strength CB, indicating that this medium may be more stimulatory than the more concentrated formulation for recovering UV-irradiated spores.

Recovery of UV-irradiated spores. Greater numbers of survivors were consistently recovered by the MPN method than by the plate count method (Table 4). Resultant D_{UV} values were as much as four times higher than those obtained by plate count recovery methods. Viability and resistance remained constant for 4 weeks. As a result of these findings, the agar plate method was no longer used in the recovery and quantification of UV-irradiated spores.

Table 5 contains resistance and stability test results for a spore suspension of strain BMT-18 (10^4 spores/ml). D_{UV} values ranged from 4.3. to 5.3 min, and survivors were recovered after exposure to a UV dose of 84,000 ergs/mm^2, dem-

TABLE 1. *Plate count recovery comparisons of nonirradiated spores of B. pumilis*

Strain and suspension lot	Recovery[a]				
	TSA	BHIA	MHA	THA	NA
BMT-18, 042778	1.5×10^8	1.1×10^8	1.5×10^8	1.4×10^8	2.5×10^7
	1.9×10^8	7.4×10^7	2.3×10^8	1.1×10^8	1.9×10^7
BMT-18, 062278	1.5×10^4	1.1×10^4	2.0×10^4	1.6×10^4	1.0×10^3
	2.0×10^4	2.2×10^4	1.7×10^4	1.8×10^4	2.7×10^3

[a] Each count represents an average of triplicate plates and is expressed as viable spores per milliliter.

TABLE 2. *Plate count and MPN recovery comparison of nonirradiated spores of B. pumilus*

Strain and lot	Recovery (viable spores/ml)				
	Plate count with TSA[a]	MPN[b]			
		TSB	BHIB	MHB	THB
BMT-18, 042778	1.5×10^8	1.1×10^8	4.6×10^7	1.2×10^8	1.1×10^8
	1.9×10^8				
	1.9×10^8				
BMT-18, 091878	1.9×10^4	2.3×10^4	1.1×10^3	2.3×10^4	1.5×10^4
E601, 012379	3.2×10^8	4.3×10^8	7.0×10^7	4.3×10^8	1.5×10^8
BMT-18, 110878	1.6×10^4	2.4×10^4	ND[c]	ND	ND
E601,[d] S41775	4.9×10^4	4.3×10^4	ND	ND	ND
	1.6×10^4	2.3×10^4	ND	ND	ND
	2.1×10^4	4.3×10^4	ND	ND	ND
	2.8×10^4	9.3×10^3	ND	ND	ND
	1.8×10^4	1.5×10^4	ND	ND	ND
	1.9×10^4	4.3×10^4	ND	ND	ND
E601,[e] 012379	3.2×10^8	1.2×10^8	ND	ND	ND
	2.9×10^8	3.9×10^8	ND	ND	ND
	3.2×10^8	4.3×10^8	ND	ND	ND

[a] Pour plate methods and TSA used to determine counts.
[b] Media were used in 2× concentrations.
[c] ND, Not done.
[d] Spores obtained by shearing six 10^4 spore strips supplied by Ethicon, Inc.
[e] Aqueous suspension of spores from *B. pumilus* E601 were used.

TABLE 3. *Comparison of TSB and CB for recovering spores irradiated with UV*

Exposure time[a] (min)	Recovery (viable spores/ml)			
	CB, 1×	TSB, 1×	CB, 2×	TSB, 2×
0 (unexposed control)	1.5×10^4	4.6×10^4	9.3×10^3	2.4×10^4
2 (12,000)	4.3×10^3	4.6×10^4	9.3×10^3	2.4×10^4
4 (24,000)	4.3×10^3	1.5×10^4	1.5×10^3	2.4×10^4
6 (36,000)	4.3×10^3	2.4×10^3	9.0×10^0	9.3×10^3
8 (48,000)	4.3×10^1	9.3×10^1	4.0×10^0	4.3×10^2
10 (60,000)	4.0×10^0	7.5×10^1	0	2.1×10^2
12 (72,000)	0	4.3×10^1	0	9.3×10^1
14 (84,000)	0	0	0	2.3×10^1
16 (96,000)	0	0	0	4.0×10^0
18 (108,000)	0	0	0	0
D_{UV}[b] (min)	2.9	3.3	2.1	3.9

[a] Time of exposure to a UV intensity of 1,000 $\mu W/cm^2$. Numbers in parentheses represent dose in ergs per square millimeter.
[b] Time required, at UV intensity of 1,000 $\mu W/cm^2$, to reduce the initial spore population by 1 logarithm (90%).

onstrating the resistance of these spores to UV. Incremental doses of UV in excess of 84,000 ergs/mm^2 were not given to spores in this study. Viable counts showed a slight decline after 6 weeks of storage; however, resistance of spores in this suspension was found to be consistent over a 6-week test period.

Resistance and recovery data for an initial spore population of 10^5 spores/ml are given in Table 6. D_{UV} determinations with 2× TSB ranged from 4.2 to 4.9 min, whereas this range with CB was 2.4 to 2.7 min. Based on the numbers of survivors recovered, the D_{UV} value for 2× TSB was greater by a factor of 1.8 than that obtained for CB. Survivors were not detected in CB MPN tubes after exposure to 84,000 ergs/mm^2, whereas some few spores dosed with 108,000 ergs/mm^2 were recovered with TSB. Counts and resistance values for this spore suspension remained stable through 12 weeks of testing.

Results from studies involving initial spore populations of 10^6/ml are listed in Table 7. The length of time required by this level of spores to absorb a sufficient dose of UV energy to cause a 1-log reduction (90%) ranged from 4.2 to 4.6 min. These D_{UV} calculations were comparable to those for both 10^4 and 10^5 spore suspensions.

TABLE 4. *UV resistance data on a B. pumilus BMT-18 spore suspension, comparing plate count and MPN recovery methods*[a]

Exposure time[b]	Recovery (viable spores/ml)					
	Initial		1 wk		4 wk	
	Plate count[c]	MPN[d]	Plate count	MPN	Plate count	MPN
0	6.5×10^3	4.6×10^3	1.2×10^4	9.3×10^3	6.7×10^3	2.4×10^3
(control)				9.3×10^3		4.3×10^3
2	5.2×10^3	4.6×10^3	5.2×10^3	2.4×10^4	3.7×10^3	2.4×10^3
(12,000)				9.3×10^3		7.5×10^3
4	2.0×10^0	2.1×10^3	1.6×10^2	2.4×10^3	7.4×10^1	4.3×10^3
(24,000)				2.4×10^3		4.3×10^3
6	2.0×10^0	7.5×10^2	0	9.3×10^2	7.4×10^1	2.4×10^3
(36,000)				4.3×10^3		2.4×10^3
8	0	2.4×10^2	0	2.4×10^2	0	9.3×10^2
(48,000)				4.3×10^2		2.4×10^2
10	0	2.3×10^1	0	2.3×10^1	0	9.3×10^1
(60,000)				4.3×10^1		2.3×10^1
12	0	0	0	2.3×10^1	0	4.3×10^1
(72,000)				1.5×10^1		1.5×10^1
14	0	0	0	0	0	4.0×10^0
(84,000)				0		0
D_{UV}[e] (min)	1.1	4.4	2.1	3.9	1.9	4.2

[a] Large-volume parent suspension (lot ST-091878-LV containing 10^4 spores/ml) were dispensed in 2-ml portions into 15-ml NDTs and irradiated at the time intervals listed.

[b] See footnote *a*, Table 3.

[c] Counts determined by agar plate method using TSA.

[d] Counts of single (initial) and duplicate (1-week and 4-week) suspensions exposed at each time interval determined by the three-tube MPN method using TSB (2×).

[e] See footnote *b*, Table 3.

TABLE 5. *UV resistance data on a B. pumilus BMT-18 spore suspension, using MPN recovery methods*[a]

Exposure time[b]	Recovery (viable spores/ml)[c]			
	Initial	2 wk	4 wk	6 wk
0	9.3×10^3	9.3×10^3	4.3×10^3	4.3×10^3
(control)	1.5×10^4	2.4×10^4	2.4×10^4	2.1×10^4
2	2.1×10^4	9.3×10^3	2.4×10^3	9.3×10^2
(12,000)	9.3×10^3	9.3×10^3	1.5×10^3	9.3×10^3
4	9.3×10^3	4.3×10^3	9.3×10^2	4.3×10^2
(24,000)	4.6×10^4	9.3×10^3	4.3×10^3	4.3×10^2
6	2.4×10^3	7.0×10^2	2.4×10^3	4.3×10^2
(36,000)	9.3×10^3	2.4×10^3	1.5×10^3	1.5×10^2
8	4.3×10^2	2.4×10^2	2.4×10^2	2.4×10^2
(48,000)	9.3×10^2	2.4×10^2	4.3×10^2	9.3×10^2
10	4.3×10^2	4.3×10^1	4.3×10^2	2.3×10^1
(60,000)	4.3×10^2	1.5×10^2	9.3×10^1	4.3×10^2
12	9.3×10^1	9.3×10^1	4.3×10^1	2.3×10^1
(72,000)	9.3×10^1	4.3×10^1	9.3×10^1	2.3×10^1
14	2.3×10^1	2.3×10^1	9.0×10^0	9.0×10^0
(84,000)	2.3×10^1	2.3×10^1	9.0×10^0	2.3×10^1
D_{UV}[d] (min)	4.5	4.3	5.0	5.3

[a] Large-volume parent suspension (lot ST-120478-LV containing 10^4 spores/ml) was dispensed in 2-ml portions into 15-ml NDTs and irradiated at the time intervals listed.

[b] See footnote *a*, Table 3.

[c] Counts of duplicate suspensions exposed at each time interval determined by the three-tube MPN method using TSB (2×).

[d] See footnote *b*, Table 3.

TABLE 6. *UV resistance data on a B. pumilus BMT-18 spore suspension, comparing CB (1×) and TSB (2×) in MPN recovery methods*[a]

Exposure time[b]	Recovery (viable spores/ml)				
	6 wk with TSB[c]	10 wk		12 wk	
		CB[d]	TSB	CB	TSB
0	1.5×10^5	7.5×10^5	2.4×10^5	4.3×10^5	2.4×10^5
(control)	7.0×10^4				
2	4.3×10^4	4.3×10^4	4.3×10^4	9.3×10^4	4.3×10^4
(12,000)	4.3×10^4				
4	2.4×10^4	2.4×10^4	4.3×10^4	9.3×10^3	4.3×10^3
(24,000)	2.4×10^4				
6	2.4×10^3	4.3×10^2	9.3×10^3	9.3×10^2	4.3×10^3
(36,000)	2.4×10^4				
8	7.1×10^3	4.3×10^2	9.3×10^3	4.3×10^2	2.4×10^3
(48,000)	2.1×10^3				
10	2.4×10^3	2.3×10^2	2.4×10^3	4.3×10^2	4.3×10^2
(60,000)	9.3×10^3				
12	4.3×10^2	9.0×10^0	2.4×10^3	2.3×10^1	2.4×10^2
(72,000)	3.0×10^2				
14	1.5×10^2	0	9.3×10^1	0	4.3×10^2
(84,000)	7.5×10^2				
16	9.3×10^1	0	2.3×10^1	0	4.0×10^0
(96,000)	4.3×10^2				
18	9.0×10^0	0	9.0×10^0	0	2.3×10^1
(108,000)	2.3×10^1				
D_{UV}[e] (min)	4.9	2.4	4.2	2.7	4.9

[a] Large-volume parent suspension (lot ST-010579-LV containing 10^5 spores/ml) was dispensed in 2-ml portions into 15-ml NDTs and irradiated at the time intervals listed.

[b] See Footnote *a*, Table 3.

[c] Counts of duplicate (6-week) and single (10-week and 12-week) suspensions exposed at each time interval determined by the three-tube MPN method using TSB (2×).

[d] Counts of single suspension (10 week and 12 week) exposed at each time interval determined by the three-tube MPN method using CB (1×).

[e] See footnote *b*, Table 3.

Surviving spores were recovered after 18 min of exposure, or a UV dose of 108,000 ergs/mm^2. Logarithmic increases in initial spore suspensions resulted in linear increases in UV doses required to destroy spores without affecting the D_{UV} values.

Table 8 is a summary of resistance data for strain E601 showing initial logarithmic reductions after 4 min of exposure. By comparison, a 90% reduction in the initial populations of BMT-18 spores was generally observed after 6 to 8 min of exposure, giving a slight shoulder in the spore destruction curve when plotted graphically. Viability and resistance of E601 spores were comparable to those of BMT-18, and this suspension was found to retain stability through 6 weeks of storage and testing.

DISCUSSION

The literature contains numerous reports on conditions which enhance the recovery of stressed or injured bacterial spores. Cook et al. (6) found that after gamma radiation, surface-spread plate counts for *B. subtilis* were greater than those obtained by an MPN tube dilution method. On the contrary, Roberts and Aldous (20) reported that recovery of UV-irradiated organisms was better on semisolid media than on conventional agar media. Nelson (18) showed that heat-treated spores of *B. subtilis* gave different counts on different agar media, and even the source of the agar has been reported to be important in the recovery process (13).

B. pumilus spores exposed to ionizing radiation have been recovered by various methods (4, 5, 10), and Zuk et al. (Annu. Meet. Am. Soc. Microbiol., 1972) described an optimal recovery procedure for *B. pumilus* E601 spores injured by gamma radiation in which CB was used. Although CB recovered higher numbers of UV-irradiated spores than agar plates in our study, it failed to compare with 2× TSB for this purpose. Some component of CB is apparently toxic to *B. pumilus* spores subjected to biocidal doses of UV. This is supported by the observation that

the 2× preparation of this medium yielded the lowest numbers of survivors in recovery tests.

TABLE 7. *UV resistance data on a B. pumilus BMT-18 spore suspension, using MPN recovery methods*[a]

Exposure time[b]	Recovery (viable spores/ml)	
	3 wk	7 wk
0	9.3×10^5	9.3×10^5
(control)	4.3×10^6	9.3×10^5
2	9.3×10^5	4.3×10^5
(12,000)	9.3×10^5	9.3×10^5
4	7.5×10^5	4.3×10^5
(24,000)	4.3×10^5	2.4×10^5
6	4.3×10^5	9.4×10^4
(36,000)	1.5×10^5	1.5×10^5
8	2.4×10^5	2.4×10^5
(48,000)	9.3×10^4	9.3×10^4
10	1.5×10^4	4.6×10^5
(60,000)	9.3×10^3	2.4×10^4
12	2.4×10^3	9.3×10^3
(72,000)	2.4×10^4	9.3×10^3
14	4.3×10^2	9.3×10^3
(84,000)	9.3×10^2	2.4×10^3
16	Not tested	2.4×10^2
(96,000)		7.0×10^2
18	Not tested	2.3×10^1
(108,000)		9.3×10^1
D_{UV}[c] (min)	4.2	4.6

[a] Large-volume parent suspension (lot ST-120479-LV containing 10^6 spores/ml was dispensed in 2-ml portions into 15-ml NDTs and irradiated at the time intervals listed. Data show counts of duplicate suspensions exposed at each time interval determined by the three-tube MPN method using TSB (2×).

[b] See footnote *a*, Table 3.

[c] See footnote *b*, Table 3.

Since it has been shown that damage to a spore by UV is of a different nature than that caused by ionizing radiation (8, 17), perhaps the repair mechanisms also differ, causing variations in nutritional requirements of spores exposed to one of these types of radiation. The more precise intricacies of these processes warrant additional study.

Certain irradiated spores become more fastidious in their nutritional demands and increasingly sensitive to the environment in which they are recovered (7, 9, 10, 23). Included are such factors as pH, oxygen, temperature, and time of incubation, each of which exerts independent influences. Speculation is not offered on the effects of incubation temperature or pH on the recovery process for UV-irradiated spores, since we investigated only a temperature range of 32 to 35°C and a pH value of 7.3 ± 0.3. Results showed that maximal numbers of positive MPN tubes were generally obtained by the 3rd day of incubation and that additional tubes did not become positive after 9 days of incubation. Therefore, a 5-day incubation period appeared adequate for the MPN test.

Slopes of spore destruction curves for gamma radiation are not influenced greatly by the initial spore population per carrier (2, 11, 16). Our data agreed with these reports as higher initial spore suspensions required greater UV doses to reduce the number of survivors, but D_{UV} computations remained relatively unchanged. Viability and resistance profiles for *B. pumilus* spores stored in glass flasks at ambient temperature were maintained for up to 12 weeks. Storage of spore suspensions in the polyethylene vials resulted in

TABLE 8. *UV resistance data on a B. pumilus E601 spore suspension using MPN recovery methods*[a]

Exposure time[b]	Recovery (viable spores/ml)			
	Initial	2 wk	4 wk	6 wk
0	9.3×10^3	4.3×10^3	2.1×10^3	2.4×10^3
(control)	4.3×10^3	9.3×10^3	4.3×10^3	4.3×10^3
2	9.3×10^3	4.3×10^3	4.3×10^3	4.3×10^3
(24,000)	4.3×10^3	1.5×10^3	4.3×10^3	2.4×10^3
4	4.3×10^2	9.3×10^2	9.3×10^2	9.3×10^2
(36,000)	4.3×10^2	9.3×10^2	2.4×10^3	1.5×10^3
6	2.3×10^2	4.3×10^2	2.4×10^2	4.3×10^2
(48,000)	9.0×10^1	2.4×10^2	9.3×10^2	2.4×10^2
8	4.3×10^1	2.4×10^2	2.4×10^2	7.0×10^2
(60,000)	4.3×10^1	7.0×10^1	2.4×10^2	2.4×10^2
10	1.5×10^1	4.0×10^0	1.5×10^2	4.3×10^2
(72,000)	9.0×10^0	2.3×10^1	1.5×10^1	1.5×10^1
12	1.5×10^0	0	9.0×10^0	1.5×10^0
(84,000)	0	0	0	0
14	0	0	0	0
(84,000)	0	0	0	0
16	0	0	0	0
(108,000)	0	0	0	0
D_{UV}[c] (min)	3.6	3.9	4.3	4.0

[a-c] See Table 7.

lowered counts and resistance characteristics.

Initial shoulders, followed by exponential death rates, were observed in spore regression curves. Strain BMT-18 exhibited a more pronounced shoulder than E601. Some spores, according to Hugo (14), possess the ability to repair damage from sublethal doses of UV, and this may account for the initial shoulder response demonstrated consistently by UV-irradiated spores of *B. pumilus*.

Greater numbers of irradiated spores were consistently recovered with 2× TSB than with TSA. Such dramatic differences were not noted between these two outgrowth media in tests involving nonirradiated spores. Perhaps as a result of diffusion, a broth medium prevents the accumulation of toxic antimetabolites from being maintained in close proximity to the damaged spore, which could ultimately be a cause of death. Lethal or antagonistic principals released from spores subjected to UV may be absorbed or bound to constituents in the broth, allowing germination, outgrowth, and recovery of damaged spores. Additionally, a broth may furnish more readily available nutrients required for repair mechanisms not immediately present in the agar.

MPN estimates were in close agreement with plate counts for determining the number of spores present in suspensions which had not been exposed to UV, allowing for a high degree of confidence in the MPN method for estimating the number of irradiated spores recovered. The inherent low precision associated with the MPN method was minimized by performing large numbers of tests.

Our results indicate that *B. pumilus* spores are resistant to UV radiation when compared with resistance data reported for other microorganisms (21, 22, 24; Schlech, unpublished data). Since efforts in the present study provided reproducible resistance and stability data, *B. pumilus* may serve as a useful biological indicator for determining and monitoring the effectiveness of UV sterilization processes. However, additional study is required before the usefulness of the recovery procedures used in this report can be fully assessed.

LITERATURE CITED

1. **American Public Health Association.** 1976. Standard methods for the examination of water and wastewater, 14th ed. American Public Health Association, Inc., New York.
2. **Berube, R.** 1977. Resistance levels of biological indicators for use in sterilization by ionizing radiation, p. 78–89. *In* E. R. L. Gaughran and K. Kereluk (ed.), Sterilization of medical products. Johnson & Johnson, New Brunswick, N.J.
3. **Borick, P. M., and M. G. Fogarty.** 1967. Effects of continuous and interrupted radiation on microorganisms. Appl. Microbiol. **5**: 785–789.
4. **Burt, M. M., and F. J. Ley.** 1963. Studies on the dose requirements for the radiation sterilization of medical equipment. I. Influence of suspending media. J. Appl. Bacteriol. **26**:484–489.
5. **Christensen, E. A., and N. W. Holm.** 1964. Inactivation of dried bacterial spores by means of ionizing radiation. Acta Pathol. Microbiol. Scand. **60**:253–265.
6. **Cook, A. M., T. A. Roberts, and J. P. Widdowson.** 1964. Gamma irradiation of *Bacillus subtilis* spores in the presence of sugars. J. Gen. Microbiol. **34**:185–193.
7. **Curran, H. R., and F. R. Evans.** 1937. The importance of enrichments in the cultivation of bacterial spores previously exposed to lethal agencies. J. Bacteriol. **34**: 179–189.
8. **Donnellan, J. E., Jr., and R. B. Setlow.** 1965. Thymine photoproducts but not thymine dimers found in ultraviolet-irradiated bacterial spores. Science **149**:308–310.
9. **Ernst, R. R.** 1968. Sterilization by heat, p. 703–740. *In* C. A. Lawrence and S. S. Block (ed.), Disinfection, sterilization, and preservation. Lea & Febiger, Philadelphia.
10. **Farkas, J., I. Kiss, and E. Andrassy.** 1967. Radiosterilization of medical products, p. 343–354. *In* Proceedings of a symposium, Radiosterilization of medical products. International Atomic Energy Agency, Vienna.
11. **Goldblith, S. A.** 1971. The inhibition and destruction of the microbial cell by radiations, p. 283–305. *In* W. B. Hugo (ed.), Inhibition and destruction of the microbial cell. Academic Press Inc., New York.
12. **Gordon, R. E., W. C. Haynes, and C. Hor-Nay Pang.** 1973. The genus Bacillus. Agriculture Handbook no. 427. Government Printing Office, Washington, D.C.
13. **Harris, N. D.** 1961. Discussion, p. 208–209. *In* Recent developments in the sterilization of surgical material, report of a symposium. Pharmaceutical Press, London.
14. **Hugo, W. B.** 1971. The destruction of bacterial spores, p. 451–612. *In* W. B. Hugo (ed.), Inhibition and destruction of the microbial cell. Academic Press Inc., New York.
15. **Kereluk, K.** 1977. Microbiological control of sterilization processes, p. 43–68. *In* E. R. L. Gaughran and K. Kereluk (ed.), Sterilization of medical products. Johnson & Johnson, New Brunswick, N.J.
16. **Morgan, B. H., and J. M. Reed.** 1954. Resistance of bacterial spores to gamma irradiation. Food Res. **19**: 357–366.
17. **Moseley, B. E. B.** 1968. Repair of damaged DNA in irradiated bacteria. Adv. Microb. Physiol. **2**:173–194.
18. **Nelson, F. E.** 1943. Factors which influence the growth of heat-treated bacteria. I. A Comparison of four agar media. J. Bacteriol. **45**:395–403.
19. **Pepper, R. E., N. T. Buffa, and V. L. Chandler.** 1956. Relative resistance of microorganisms to cathode rays. III. Bacterial spores. Appl. Microbiol. **4**:149–152.
20. **Roberts, R. B., and E. Aldous.** 1949. Recovery from ultraviolet radiation in *Escherichia coli*. J. Bacteriol. **57**:363–375.
21. **Rubbo, S. D., and J. F. Gardner.** 1965. Sterilization by radiation, p. 78–96. *In* S. D. Rubbo and J. F. Gardner (ed.), A review of sterilization and disinfection. Yearbook Medical Publishers, Inc., Chicago.
22. **Sykes, G.** 1958. Radiation sterilization, p. 138–149. *In* H. M. Bunbury (ed.), Disinfection and sterilization. D. van Nostrand Co., Princeton, N.J.
23. **Williams, O. B., and J. M. Reed.** 1941. The significance of the incubation temperature of recovery cultures in determining spore resistance to heat. J. Infect. Dis. **71**: 225–2257.
24. **Zelle, M. R., and A. Hollaender.** 1955. Effects of radiation on bacteria, p. 365–430. *In* A. Hollaender (ed.), Radiation biology, vol. 2: Ultraviolet and related radiations. McGraw-Hill Book Co., New York.