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Isolation of Recombination-Defective and UV-Sensitive Mutants of *Bacillus megaterium*

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Mutants of *Bacillus megaterium* QMB1551 sensitive to mitomycin C or methyl methanesulfonate were isolated and characterized phenotypically. Cell survival after UV-light and gamma-ray exposure was determined, as was transductional recombination. Of the mutants tested, three were sensitive to UV but remained recombination proficient. The UV-sensitive mutants were also reduced in host cell reactivation. At least three mutants had undetectable transduction frequencies, i.e., less than 0.3 to 1.3% of the parental strain frequencies, and so appear to be recombination deficient. Sensitivities of these mutant strains to UV light and gamma radiation were compared with those of parental *B. megaterium* as well as parental, *recE4*, *recA1*, *uvrA19*, and *uvrB109* strains of *Bacillus subtilis*. In each case, the strains of *B. megaterium*, including the parental strains, showed a higher percentage of cell survival than *B. subtilis*.

The genes involved in procaryotic recombination and repair have been characterized most extensively for *Escherichia coli* (see recent reviews in references 13 and 24). Repair systems in this species include photoreactivation, excision repair, postreplication repair, and the inducible SOS response. Many of the genes involved in repair also function in recombination. The characterization of similar systems in the gram-positive organisms is less well developed (16) but is already revealing both similarities and differences in comparison with *E. coli*. With *Bacillus subtilis*, several mutants deficient in recombination or repair have been isolated and characterized (5, 7, 10, 11). Recombinational events, including both heterologous and homologous transformation and transduction, prophage induction, and the detection of donor-recipient complex formation during transformation (6, 10), have all been used to classify recombination-deficient (*rec*) mutants of *B. subtilis*. At least 40 *rec* mutations have been placed into 10 genes at 9 different loci (5, 15, 19). The *recE4* mutant strains of *B. subtilis* were the most UV sensitive and were depressed at least 1,000-fold in transformation frequency (7). Recently, deVos and Venema (3) detected a 45,000-dalton protein, missing in *recE4* mutants, that has been shown to be induced by UV irradiation or mitomycin C. The *B. subtilis recE4* gene can be complemented by the *recA* genes of *E. coli* (2) and *Proteus mirabilis* (8). Functional similarities between the *recF* and *recG* genes of *B. subtilis* and the *E. coli recL* gene have also been found (4). Dodson and Hadden (4) have suggested that *recE4* is necessary for synapse formation, while *recF* and *recG* are required for formation and resolution of crossovers during recombination.

Differences between *E. coli* and *B. subtilis* repair pathways have also been found. For example, photoreactivation has not been described in *B. subtilis* (16), and there is a type of repair unique to the genus *Bacillus* called spore-specific repair (17). While Love and Yasbin (14) have shown that there is an inducible repair system (SOB) in *B. subtilis* analogous to the SOS repair system of *E. coli*, it may be more complicated, requiring *recE4*, *recA1*, *recB*, *recG*, or an unlinked *tsi-23* mutation for induction. Mutants have been isolated that lack an ATP-dependent DNase activity, but this activity probably does not play a significant role in recombination in *B. subtilis* (9). Although the excision repair (Uvr) system of *B. subtilis* is similar to that of *E. coli* in that it is not specific for thymine dimers, unlike the system in *E. coli*, the *Bacillus* inducible Weigle reactivation system is specific for pyrimidine dimers (9).

Very little has been reported on repair or recombination genes in other bacilli, although a mutant of *B. cereus* that may be similar to *uvrD* mutants of *E. coli* has been reported (25). We have begun to isolate mutants of *B. megaterium* deficient in recombination or repair. We were interested in investigating the stability and expression of plasmids carrying foreign DNA, including some carrying potentially homologous *B. subtilis* genes. *B. megaterium* is a sporeformer that shows only 8% DNA-DNA hybridization with *B. subtilis* (21) and may prove to be an advantageous host for studying the expression of *B. subtilis* genes. Since stability of plasmids carrying homologous DNA has been a significant problem in *B. subtilis* Rec^+ hosts, it is possible that, for plasmids carrying some *B. subtilis* genes, stability will also be increased in *B. megaterium* by using a Rec^- host. Availability of *B. megaterium* recombination mutants might then facilitate cloning in this organism. In addition, *B. megaterium* recombination and repair mutants should help to determine whether the repair and recombination pathways of this species are similar to those of other gram-positive organisms. This report describes the isolation of three possible recombination-deficient mutants and three other UV-sensitive mutants and gives their initial characterization.

MATERIALS AND METHODS

Strains and culture conditions. The strains used in this study are listed in Table 1. All *B. megaterium* strains are derivatives of strain QMB1551. Routine growth of *B. megaterium* and preparation of lysates were done in supplemented nutrient broth (22) containing (per liter) 8 g of nutrient broth (Difco Laboratories) 1 g of glucose, 13.4 mM KCl, 0.02 mM $MnCl_2$, 1 μM $FeSO_4$, 1 mM $MgSO_4$, and 1 mM $CaCl_2$. Minimal medium (12) contained (per liter) 5 g of glucose, 2 g of $(NH_4)_2SO_4$, 14 g of K_2HPO_4, 1 g of trisodium citrate · $2H_2O$, 0.2 g of $MgSO_4$ · $7H_2O$, 3.6 μM $FeCl_2$, 0.1 mM $MnCl_2$, and 0.1 mM $CaCl_2$. For transductions, the Fe, Mn, and Ca salts were omitted, and the pH was adjusted to pH 6.5 after

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TABLE 1. Strains used in this study

Strain	Genotype	Source[a]
B. megaterium		
QMB1551	Prototrophic	J. C. Vary
JV78	*leuC4 str-3*	J. C. Vary
JV69	*metA3*	J. C. Vary
PV250	*leuC4 str-3 uvr-1*	This study
PV260	*leuC4 str-3 rec-1*	This study
PV271	*leuC4 str-3 rec-2*	This study
PV275	*metA3 uvr-2*	This study
PV278	*leuC4 str-3 uvr-3*	This study
PV280	*leuC4 str-3 rec-3*	This study
B. subtilis		
DBS80	*trpC2*	D. Dean
1A43	*trpC2 recA1*	BGSC
1A46	*trpC2 thr-5 recE4*	BGSC
1A346	*hisH2 thyA1 thyB1 uvrB109*	BGSC
1A374	*trpC2 thyA1 thyB1 uvrA19*	BGSC

[a] J. C. Vary, University of Illinois School of Medicine at Chicago; D. Dean, Ohio State University, Columbus, Ohio; BGSC, *Bacillus* Genetic Stock Center, Ohio State University, Columbus, Ohio.

autoclaving. The bacteriophage was diluted in phage buffer (1). *B. megaterium* was grown at 30°C with shaking at 300 rpm. *B. subtilis* was grown at 37°C in Penassay broth (Difco) with shaking at 300 rpm. Single colonies from an overnight culture were inoculated into 250-ml flasks containing 25 ml of supplemented nutrient broth or Penassay broth plus 40 μg of each auxotrophic requirement per ml. Optical densities were determined on a Bausch & Lomb Spectronic 20 spectophotometer at a wavelength of 660 nm.

Phage propagation was as described previously (23). Lysates contained 10^8 to 10^{11} PFU/ml. For transductions, recipient cells at an A_{660} of 0.8 to 1.0 were washed and spread at a multiplicity of infection of 1 on selective plates containing phage already exposed to 168 J/m^2 of 254-nm UV light.

Isolation of mutants. Two parental strains, JV69 and JV78, were grown to an A_{660} of 1.0 in supplemented nutrient broth, and 20-ml samples were mutagenized with *N*-methyl-*N'*-nitrosoguanidine (100 μg/ml for 30 min) before being washed and separated into 2-ml cultures for growth at 30°C overnight with shaking. Each culture was then diluted, plated for single colonies on supplemented nutrient broth, and incubated overnight at 30°C. The colonies were replica plated onto supplemented nutrient broth plates containing 0.25 μg of mitomycin C (Sigma Chemical Co.) per ml or 0.45 μg of methane methylsulfonate (Sigma) per ml as well as onto control media. Colonies which failed to grow in the presence of mitomycin C or methane methylsulfonate were purified and characterized further. A total of 53 cultures was screened. Only one sensitive isolate was saved from each culture to avoid the possible isolation of siblings.

UV-light and gamma-ray sensitivity. To test sensitivity to UV irradiation, cells were grown to an A_{660} of 0.8 to 1.0 (late logarithmic phase). A 5-ml sample was washed and suspended in an equal volume of minimal medium broth without required supplements. A 2-ml sample was placed in the bottom half of a glass petri dish (13 by 50 mm) and was exposed with constant stirring to UV light from a General Electric G8/T5 source (254 nm) at 1.5 J/m^2/s. Energy was measured with a Blak-Ray UV-light meter calibrated frequently against an International light 1500 research radiometer. Cells were removed at various intervals after exposure, diluted, and plated for single colonies. All manipulations were performed under red light, and all plates were kept in the dark until counted. Incubations were at 30°C for 12 to 24 h. The gamma-ray source was a ^{137}Cs gamma meter that emitted approximately 20 kR of energy per h. Cells were prepared as described for UV irradiation. Since these tests took up to 6 h, a control was always run in which cells not exposed to radiation and maintained under the same conditions were counted initially and at the end.

Host cell reactivation and efficiency of plating. Lysates of phage MP13 (about 10^{10} PFU/ml) were diluted 100-fold in phage buffer and were UV irradiated with stirring at either 4 or 20 J/m^2. The phage was then diluted and plated in soft-agar overlays on various mutant strains to test their ability to repair the phage DNA. Titers for unirradiated phage were also determined with mutants and parental strains as controls to determine the efficiency of plating of the phage on each strain.

Revertant isolation. To isolate mitomycin C-resistant revertants, the *rec* strains were grown in supplemented nutrient broth to an A_{660} of 0.7 to 0.9, and 0.1 ml was spread on supplemented nutrient broth plates containing 0.25, 0.3, or 0.5 μg of mitomycin C per ml. After 48 h of incubation at 30°C, colonies were picked to supplemented nutrient broth plates, grown overnight, and then replica plated to plates with the same concentrations of mitomycin C. All resistant colonies were then purified and characterized further.

RESULTS

Isolation and screening of mitomycin C- and methane methylsulfonate-sensitive mutants. Thirty-nine mutants sensitive to mitomycin C or methane methylsulfonate or both were isolated from approximately 5,000 colonies tested. Four mutants were derivatives of strain JV69 (*metA3*); the rest were derivatives of JV78 (*leuC4 str-3*). To screen for recombination and repair capabilities, each mutant strain and its parent was grown to an A_{660} of about 0.8 before exposure to UV light. Each strain was diluted and plated, as described in Materials and Methods, both before and after exposure to 180 J/m^2 of UV irradiation. In addition, each parent and mutant strain was transduced for either Leu$^+$ or Met$^+$ with MP13 grown on prototrophic strain QMB1551 and was screened for the ability to propagate MP13 phage. Most of the mutants exhibited no enhanced UV sensitivity when compared with their parental strains and could be transduced at levels similar to those of the parental strains, although seven strains transduced at 27 to 49% of parental strain frequencies. Six mutants were shown by screening to be more UV sensitive than parental strains, and three of these were very reduced in transducing ability. These six mutants were further characterized.

Transduction of UV-sensitive strains. Each of the UV-sensitive strains was transduced with phage MP13 grown on strain QMB1551, and their frequencies of transduction were compared with those of parental strains as shown in Table 2. As the data show, three of the mutants were transduced by MP13 at near parental levels. Since these three mutants were proficient in transduction but were UV sensitive, they were tentatively designated *uvr* mutants. Three other mutants had undetectable levels of transduction. However, a decrease in transducing ability might be caused by a decreased ability to adsorb or propagate the phage, so all six mutants were tested quantitatively for efficiency of plating of MP13. Since the phage plating efficiencies were from 83 to 94% of parental values, the greatly reduced frequencies of transduction cannot be explained by an inability to propagate MP13.

TABLE 2. Transduction of the UV-sensitive strains

Strain	Marker selected	Frequency of transduction (no. of tests)[a]	Percentage of parental sensitivity	MP13 EOP[b]
JV78 *rec*$^+$	Leu$^+$	5.6×10^{-7} (6)	100	1.00
PV260 *rec-1*	Leu$^+$	$<7.6 \times 10^{-9}$ (7)	<1.35	0.86
PV271 *rec-2*	Leu$^+$	$<1.8 \times 10^{-9}$ (4)	<0.31	0.94
PV280 *rec-3*	Leu$^+$	$<3.5 \times 10^{-9}$ (4)	<0.62	0.83
JV78 *uvr*$^+$	Leu$^+$	1.2×10^{-6} (2)	100	1.0
JV69 *uvr*$^+$	Met$^+$	9.0×10^{-7} (2)	100	1.0
PV250 *uvr-1*	Leu$^+$	7.4×10^{-7} (2)	61	0.64
PV275 *uvr-2*	Met$^+$	7.9×10^{-7} (2)	88	0.80
PV278 *uvr-3*	Leu$^+$	8.3×10^{-7} (2)	69	1.0

[a] Transduction frequency with phage MP13 is expressed as transductants per PFU and is the average of the number of tests indicated in parentheses.
[b] EOP, Efficiency of plating.

Therefore, mutants PV260, PV271, and PV280 have been tentatively designated as *rec*, or deficient in recombination.

Further characterization of the UV-sensitive mutants. The sensitivity to UV irradiation of the three possible *uvr* mutants was compared with sensitivities of parental *B. megaterium* and with *B. subtilis* wild type and *uvr* mutants

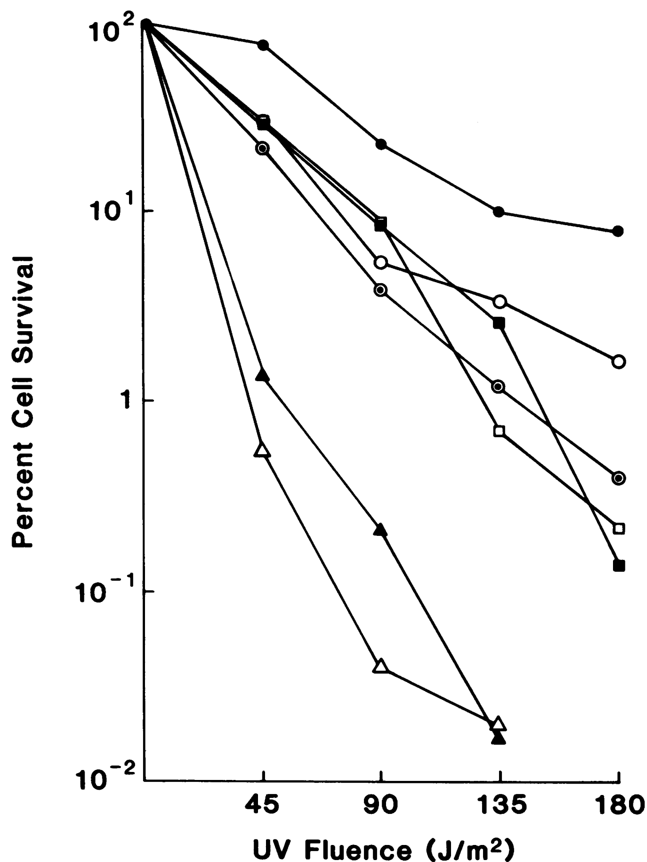

FIG. 1. UV survival of the *B. megaterium* wild type and putative *uvr* mutants compared with *B. subtilis uvr*$^+$ strains. Washed mid-logarithmic-phase cells were exposed with stirring to UV (1.5 J/m^2 per s) for different times and then were diluted, plated, and incubated in the dark. *B. megaterium strains*: ●, JV78 *uvr*$^+$; □, PV278 *uvr-1*; ■, PV250 *uvr-2*; ⊙, PV275 *uvr-3*. *B. subtilis* strains: ○, DBS80 *uvr*$^+$; △, 1A374 *uvrA19*; ▲, 1A346 *uvrB109*.

(Fig. 1). It is evident from Fig. 1 that mutants PV250, PV275, and PV278 are all more sensitive to UV irradiation than the parental strain. The sensitivities of these strains were not as great as that seen in the *B. subtilis uvr* strains tested. This difference can be seen at 135 J/m^2, at which survival of *uvrA19* and *uvrB109* cells was 0.6 and 0.53%, respectively, of parental survival compared with survival rates of 7 to 26% of parental survival at the same fluence for *B. megaterium* mutants *uvr-1*, *uvr-2*, and *uvr-3*.

It should be pointed out that there is a distinct shoulder on the parental *B. megaterium* curve. This shoulder is also evident in curves for both parental strains and *rec* mutants (Fig. 2 and 3) with both UV and gamma-ray sources but is absent in curves for the *uvr* mutants represented in Fig. 1. To test the ability of the three UV-sensitive mutants and three *rec* mutants to carry out host cell reactivation, these mutants were infected with MP13 phage that had been exposed to either 4 or 20 J/m^2 of UV irradiation as described in Materials and Methods. The data in Table 3 show that while the three *rec* mutants are capable of host cell reactivation near parental levels, all three *uvr* strains were reduced to 33 to 58% of wild-type ability. The reduction was more apparent at 4 J/m^2.

The three mutants deficient in the ability to act as recipients for transducing DNA were also tested along with the *rec*$^+$ parent for sensitivity to both UV light and gamma radiation (Fig. 2 and 3). All three *rec* strains were more UV sensitive than the parental strain (Fig. 2), but were less UV sensitive than the *uvr* mutants represented in Fig. 1. The difference in sensitivity was not nearly as great as that seen between the *B. subtilis rec*$^+$ and *recE* or *recA* strains. Again, the greater resistance of *B. megaterium* to UV light than *B. subtilis* and the prominent shoulder were evident. The sensitivity of all the *rec* strains to gamma rays was tested. The *B. megaterium rec* mutants were characteristically also sensitive to gamma radiation, but all three mutants were more resistant to radiation than the *rec* mutants of *B. subtilis* (Fig. 3).

Since the effects of mitomycin C, methane methylsulfonate, UV and gamma-ray sensitivities, and recombination deficiencies might be the result of multiple lesions rather than a single mutation, mitomycin C-resistant revertants of the *rec* strains were isolated as described in Materials and Methods and were tested for the phenotypic characteristics shown in Table 4. Reversion to mitomycin C resistance simultaneously caused reversion to methane methylsulfonate resistance (data not shown) and UV resistance in mutant PV260-R1. For all three *rec* mutants, we were able to

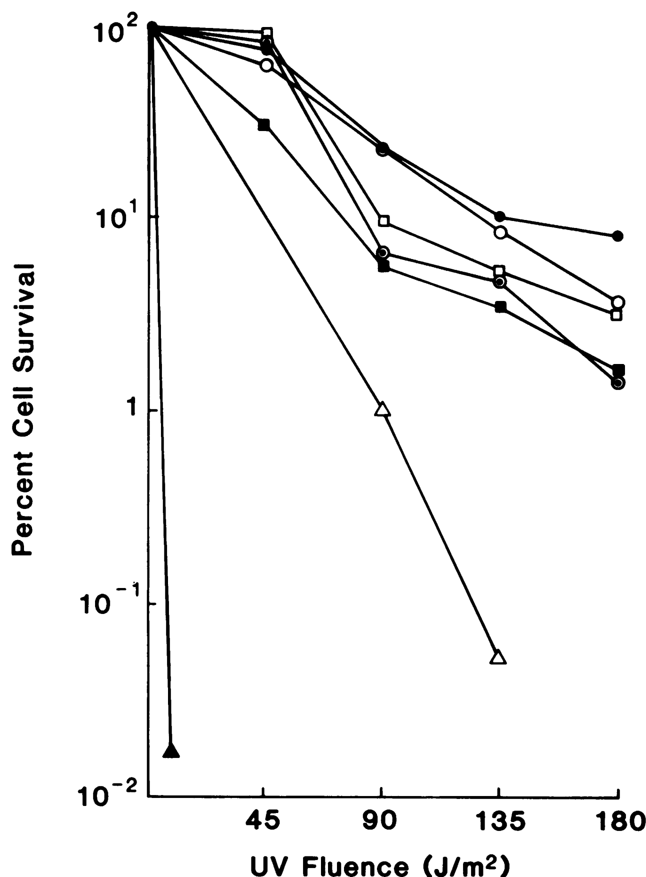

FIG. 2. UV survival of *B. megaterium* wild type and putative *rec* mutants compared with *B. subtilis rec*$^+$ and *recE4* and *recA1* mutants. Conditions were the same as for Fig. 1. *B. megaterium* strains: ●, JV78 *rec*$^+$; □, PV260 *rec-1*; ⊙, PV271 *rec-2*; ○, PV280 *rec-3*. *B. subtilis* strains: ■, DBS80 *rec*$^+$; △, 1A43 *recA1*; ▲, 1A46 *recE4*.

isolate revertants that had also partially recovered their ability to be transduced by MP13.

DISCUSSION

We isolated several mitomycin C- and methyl methanesulfonate-sensitive mutants of *B. megaterium*. Of these mutants, six were shown to be move UV sensitive than the wild type in initial screening tests and were investigated further. Three mutants, designated *uvr-1*, *uvr-2*, and *uvr-3*, were also reduced in their capacity for host cell reactivation of MP13 UV-damaged DNA. This result is consistent with the phenotypes of excision repair mutants of both *E. coli* and *B. subtilis*. Host cell reactivation has not been measured in all *B. subtilis uvr* mutants. However, Sadaie and Kada (20) reported that host cell reactivation in *uvr-19* was reduced to 9% of the wild-type level, while Yasbin (26) reported 2% of wild-type levels for mutant *uvr-1* at 50 J/m^2. In the same study, Yasbin (26) also detected 69% host cell reactivation in a *polA5* mutant strain. It is possible that our Uvr mutants may be *polA* mutants, since they exhibited 33 to 58% reactivation compared with wild type. However, the host cell reactivation phenotype is dependent on the phage used in *B. subtilis*. We used a phage specific for *B. megaterium*, and so a direct comparison of the degree of host cell reactivation may not be possible.

The mutations in strains PV260, PV271, and PV280 have been designated *rec-1*, *rec-2*, and *rec-3*, respectively, since they cause increased mitomycin C, methane methylsulfonate, UV, and gamma-ray sensitivities and greatly reduce the ability to act as recipient for transducing DNA. When the sensitivities to both UV and gamma radiation of *B. megaterium* and *B. subtilis rec* mutant strains were compared, it was observed that *B. subtilis recA* and *recE4* mutants were slightly less sensitive to gamma rays than to UV radiation and consistently exhibited a change in sensitivity to gamma radiation above 20 kR. No comparable change in sensitivity was observed in the curves for *B. megaterium rec* mutant strains. However, *B. megaterium* was consistently more resistant to UV and gamma irradiation than *B. subtilis*. Although the mutants in this study were more resistant than the *B. subtilis* mutants tested, some of their lack of sensitivity may be because of an overall resistance of the species itself. To our knowledge, this is the first report of the effect of radiation on *B. megaterium* vegetative cells in comparison with another species of *Bacillus*. The shoulder evident in all parental and *rec* mutant survival curves could be caused by several phenomena

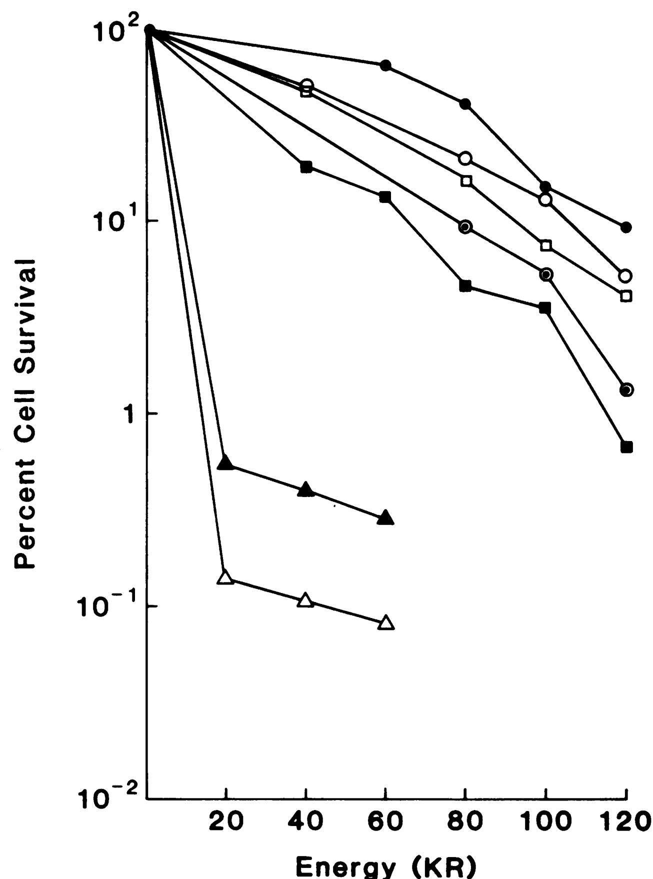

FIG. 3. Gamma-ray survival of *B. megaterium* parental and *rec* mutant strains compared with *B. subtilis rec*$^+$ and *recE4* and *recA1* mutant strains. Cells in late logarithmic phase were washed and exposed to a gamma-ray source (^{137}Cs, 20kR/h) for various times up to 6 h. Cells were withdrawn, diluted, plated, and incubated overnight. Controls showed no change in survival in the same media without autotrophic requirements. *B. megaterium* strains: ●, JV78 *rec*$^+$; ○, PV260 *rec-1*; ⊙, PV271 *rec-2*; □, PV280 *rec-3*. *B. subtilis* strains: ■, DBS80 *rec*$^+$; ▲, 1A43 *recA1*; △, 1A46 *recE4*.

TABLE 3. Capacity for host cell reactivation[a]

Strain	PFU ($\times 10^7$) per ml after exposure of phage MP13 to UV at:		PFU ($\times 10^8$) per ml after exposure of phage MP13 to UV at:	
	0 J/m²	4 J/m² (% survival)	0 J/m²	20 J/m² (% survival)
JV78 *uvr*⁺ *rec*⁺	62	23 (37)	70	8.0 (12)
PV260 *rec-1*	59	21 (35)	61	6.2 (10)
PV271 *rec-2*	57	19 (34)	57	5.4 (9)
PV280 *rec-3*	53	18 (35)	53	4.7 (9)
JV78 *uvr*⁺ *rec*⁺	12	5.2 (42)	70	8.0 (12)
JV69 *uvr*⁺ *rec*+	ND[b]	ND	61	7.4 (12)
PV250 *uvr-1*	29	4.0 (14)	34	1.9 (6)
PV275 *uvr-2*	35	7.7 (22)	35	2.3 (7)
PV278 *uvr-3*	2.1	0.3 (15)	40	2.7 (7)

[a] MP13 phage that had been exposed to different fluences of UV light were then diluted and plated onto the strains shown to test for the capacity of each strain to repair the phage DNA. The data represent three experiments from among at least three experiments performed at each fluence for each mutant. The data for 4-J/m² exposure are from two separate experiments, with one for the *rec* mutants and another for the *uvr* mutants. The data for 20-J/m² exposure are from the same experiment.

[b] ND, Not determined.

including increased target size (more DNA), increased chaining of cells, or a more efficient repair system. The disappearance of the shoulder in the survival curves for the *uvr* mutants suggests that increased repair capability in *B. megaterium* may at least contribute to its increased resistance. During these studies it was also observed that the parent strain and mutants often formed filaments after exposure to mitomycin C. This induced filamentous growth may indicate induction of an SOB repair pathway in *B. megaterium* similar to that described by Love and Yasbin (14) for *B. subtilis*.

The three *rec* mutants we isolated all had undetectable transduction frequencies. This deficiency in ability to form transductional recombinants was shown not to be caused by reduced propagation or adsorption of the transducing phage, since all three mutants could support phage infection at near wild-type efficiencies. For *B. subtilis*, Dubnau et al. (7) reported that the homologous transduction frequencies of the *rec* mutants tested ranged from 87% of wild type for *recA1* to less than 0.036% for *recE4*. Our *B. megaterium rec* mutants had transduction frequencies that were less than 0.3 to 1.3%. It cannot be determined at this time which gene loci in *B. subtilis* correspond to *rec-1*, *rec-2*, and *rec-3*, since preliminary mapping data have so far been negative (data not shown). We are trying to clone the *rec* genes of *B. megaterium* into *B. subtilis* to find possible complementation of *B. subtilis* genes with those of *B. megaterium*. What is likely is that all are point mutants, since all three mitomycin C-sensitive strains reverted to mitomycin C resistance and showed partial recovery of recombination proficiency simultaneously. As with *B. megaterium*, it has been very difficult to isolate revertants of *rec* mutants of *B. subtilis* that have regained full recombinational ability (18). Okubo and Romig (18) reported that if they increased the levels of mitomycin C, they could isolate better revertants. In our attempts to isolate *B. megaterium rec*⁺ revertants, we increased mitomycin C from 0.25 to 0.5 μg/ml with no increased success. Since full recovery of recombinational ability was not observed in our revertants, the possibility exists that these revertants may be the result of inefficient suppressor mutations, perhaps analogous to *sbcA* or *sbcB* mutations in *E. coli*.

TABLE 4. Phenotypes of *rec* strains and their revertants[a]

Strain and revertants	Response to: MitC[c]	Response to: UV	Transduction (% parental)	MP13 EOP[b]
JV78	R	R	100	1.00
PV260	S	S	<1	0.88
R1	R	R	43	0.61
R2	R	R/S	9	0.96
R3	R	R/S	18	0.91
PV271	S		<1	0.82
R1	R	R/S	16	1.00
R2	R	R	22	0.94
R3	R	ND[d]	4	ND
PV280	S	R	<1	0.76
R1	R	R	9	0.85
R2	R	R	7	0.93

[a] Revertants (R1, R2, R3, etc.) for each *rec* mutant strain were selected on plates containing from 0.25 to 0.50 g of mitomycin C (MitC) per ml and were purified and tested for spontaneous recovery of UV resistance and ability to serve as transduction recipients. Strain QMB1551 was donor for the transductions. The efficiency of plating of the phage on each recipient was determined at the same time.

[b] EOP, Efficiency of plating.

[c] R, Resistant; S, sensitive; R/S, slightly more sensitive than parental strain.

[d] ND, Not determined.

Nevertheless, the availability of three mutants defective in recombination now makes it possible for us to further test the stability of plasmids carrying *Bacillus* DNA in this species. For this purpose, and for further investigation of recombination and repair in *B. megaterium*, the *uvr* and *rec* mutations reported in this study should prove useful.

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