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Effect of Repair Deficiency and R Plasmids on Spontaneous and Radiation-Induced Mutability in *Pseudomonas aeruginosa*

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The effect of R plasmids on spontaneous and radiation (ultraviolet and gamma)-induced mutability in *Pseudomonas aeruginosa* was studied in strains containing the radiation-sensitive markers *polA3* or *rec-2* and the revertable auxotrophic markers *hisO27* and *trpB1*. In the absence of an R plasmid, the radiation-induced mutability was dependent on the *recA*$^+$ genotype and independent of the *polA*$^+$ genotype, whereas spontaneous mutability was similar in all genetic backgrounds. R plasmids pPL1, R2, and pMG15 increased the ultraviolet radiation survival and ultraviolet-induced mutability of wild-type and *polA* host cells but did not alter either effect in a *recA* mutant. These R plasmids also increased the gamma radiation survival and gamma-induced mutability of wild-type host cells but did not alter either effect in a *polA* or *recA* mutant. R plasmids pPL1, R2, and pMG15 also enhanced the level of spontaneous mutagenesis in wild-type host cells but not in a *polA* or *recA* mutant. These data suggested that a common plasmid gene product(s) may participate in various *recA*-dependent, error-prone deoxyribonucleic acid repair pathways of *P. aeruginosa*. The properties of a mutant R plasmid, pPL2, originally selected because it lacked enhanced ultraviolet-induced mutability, supported this conclusion.

Some R plasmids (17, 18) and FP plasmids (22) of *Pseudomonas aeruginosa* can decrease the UV light sensitivity of host cells (UV protection) and enhance the UV-induced mutability of several auxotrophic markers in *P. aeruginosa*. This suggests that these plasmids contribute to the error-prone repair processes of the organism. Furthermore, studies of repair-deficient mutants of *P. aeruginosa* containing various R plasmids (17, 18), as well as similar studies of *Escherichia coli* (23, 24) and *Salmonella typhimurium* (21) with R plasmid R46 (and its derivatives), indicate that plasmid-mediated UV protection is dependent on a *recA*$^+$ genotype, not on the presence of the *uvr*$^+$ or *polA*$^+$ genotype.

This study was initiated to determine whether there is a similar genotypic dependence of R-plasmid-enhanced spontaneous and radiation-induced mutability in *P. aeruginosa*. To facilitate this study, one strain containing the revertable auxotrophic markers *hisO27* and *trpB1*, as well as the previously defined *rec-2* allele (4), and a second strain containing the same revertable auxotrophic markers and the *polA3* allele (15) were constructed. Cells carrying the *polA3* allele are phenotypically similar to *polA* mutants of *E. coli* (7). They show increased sensitivity to UV and gamma irradiation and treatment with methyl methanesulfonate or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, as well as a reduced ability to reactivate phage E79 treated with UV or methyl methane sulfonate (15). Cells carrying the recombination-deficient mutant allele *rec-2* (4) are phenotypically similar to the *recA* mutants of *E. coli* (5).

The survival of repair-proficient and repair-deficient strains after UV and gamma irradiation in the presence or absence of the UV-protecting R plasmids pPL1 (18), R2 (17), and pMG15 (17) was determined. The levels of spontaneous and radiation-induced back-mutations to prototrophy of the auxotrophic markers *hisO27* and *trpB1* were also determined for these strains.

MATERIALS AND METHODS

Bacteria. All of the bacterial strains used in this study are derived from wild-type *P. aeruginosa* PAO1 (ATCC 15692) (10). The bacterial strains and plasmids used in this study are listed in Table 1. Bacterial strain GMC146 was constructed by FP2 conjugation and F116 transduction. The *polA3* derivative of GMC146 was selected after FP2 conjugation, as an *ilv*$^+$, methyl methane sulfonate-sensitive recombinant. The *rec-2* derivative of GMC146 was selected after pMG15 conjugation, as an *ilv*$^+$, methyl methane sulfonate-sensitive recombinant. Strain GMC154 was selected as an *ilv*$^+$, methyl methane sulfonate-resistant recombinant

TABLE 1. *Bacterial strains and plasmids used*

Strain/plasmid	Characteristic(s)[a]	Reference
P. aeruginosa		
TRPB1	*trpB1*	6
GMA027	*hisO27*	20
PA0170	*leu-38* FP	14
GMC146	*trpB1 hisO27 ilv-202 str-1*	This paper
GMC154	*trpB1 hisO27 str-1*	This paper
GMC156	*trpB1 hisO27 rec-2 str-1*	This paper
GMC160	*trpB1 hisO27 polA3 str-1*	This paper
GMC164	*hisO27 str-1*	This paper
Plasmid		
pPL1	Gm Sm Su Hg Phi⁻ E79 UV	18
R2	Cb Sm Su UV	11
pMG15	Hg Cma UV	8
pPL2	Gm Sm Su Hg Phi E79 UV	16

[a] Antibiotic resistances: Cb, carbenicillin; Gm gentamicin; Sm, streptomycin; Su, sulfonamide. Hg, Resistance to mercuric ions; Phi, interference with phage propogation; UV, UV protection or resistance; Cma, chromosome-mobilizing ability (9).

and is considered to be *polA3*$^+$ *rec-2*$^+$ (wild type with respect to repair).

General culture procedures and plasmid transfer. General culture procedures and plasmid transfer techniques have been previously described (13, 14, 18).

UV irradiation. The source of UV radiation was a 15-W germicidal lamp (no. 15T8, General Electric Co.) shielded to give 0.8 to 0.9 J/m^2 per s at 48 cm. In the case of low doses, a Philips (TUV) tube giving 0.1 J/m^2 per s at 40 cm was used.

To determine UV survival, 0.01-ml drops from appropriate dilutions of an overnight (16-h) 37°C shaken nutrient broth culture were spotted onto the surface of nutrient agar plates. After the drops had dried, the plates were exposed to various doses of UV radiation. Colonies were counted after overnight incubation at 37°C. To determine the yields of a *trp*$^+$ or *his*$^+$ back-mutation in the strains tested, saline-washed cell suspensions (ca. 10^8 to 10^9 cells per ml) were prepared from an overnight (16-h) 37°C shaken nutrient broth culture. Samples (0.1 ml) of irradiated and control cell suspensions were spread on minimal agar medium (13) plus nutrient broth (Oxoid CM67, 2.5% [vol/vol]), plus tryptophan (100 μg/ml) or histidine (100 μg/ml) as required. The level of back-mutation in control and UV-irradiated cell suspensions was determined by using three replicate samples plated on appropriate media and incubated at 37°C for 3 days. In these experiments, UV survival was determined by plating appropriate dilutions on minimal agar medium plus nutrient broth (Oxoid CM67, 2.5% [vol/vol]), with tryptophan (100 μg/ml) or histidine (100 μg/ml) as appropriate.

Gamma irradiation. Bacteria were irradiated with a ^{60}Co source at a dose rate of 800 rads/min. Saline-washed cell suspensions were prepared as for UV irradiations. Yields of cells with *trp*$^+$ and *his*$^+$ back-mutations and survivors after irradiation were obtained as described above.

Spontaneous mutability. To measure spontaneous mutation frequencies, 10 individual broth cultures were inoculated with 40 to 100 cells of the strain to be tested. Each culture was incubated overnight (16 h) at 37°C with shaking. Duplicate 0.1-ml samples of saline-washed cell suspensions (ca. 10^8 to 10^9 cells per ml) were spread on minimal agar medium plus nutrient broth (Oxoid CM67, 2.5% [vol/vol]) plus tryptophan (100 μg/ml) or on minimal agar medium plus nutrient broth (Oxoid CM67, 2.5% [vol/vol]) plus histidine (100 μg/ml) and the plates were incubated at 37°C for 3 days. Viable cell counts for each of the cultures were determined on minimal agar medium plus nutrient broth (Oxoid CM67, 2.5% [vol/vol] plus tryptophan (100 μg/ml) or histidine (100 μg/ml) as appropriate. The number of back-mutations to *his*$^+$ or *trp*$^+$ per 10^7 cells plated was calculated for each strain.

RESULTS

Effect of repair deficiency and R plasmids on radiation sensitivity. Survival rates after UV and gamma irradiation of GMC154 *rec-2*$^+$ *polA3*$^+$, GMC160 *rec-2*$^+$ *polA3*, and GMC156 *rec-2 polA3*$^+$ are shown in Fig. 1A and B. Both GMC160 and GMC156 are more sensitive than GMC154 to the lethal effects of UV and gamma irradiation.

R plasmids R2, pPL1, and pMG15, which have previously (17, 18) been shown to decrease the UV sensitivity of host bacteria, were transferred to strains GMC154, GMC156, and GMC160. These plasmids were selected as representative of the various plasmid incompatibility groups of *P. aeruginosa* and as members of two classes of UV-protecting plasmids defined on the basis of their ability to protect (R2 and pMG15) or not protect (pPL1) against UV in the presence of sublethal concentrations of sodium arsenite (1 mM) (17; unpublished data). The basis of this difference in response to UV irradiation in the presence of sodium arsenite is not known.

Survival rates after UV and gamma irradiation of strains GMC154, GMC156, and GMC160 carrying the R plasmids are shown in Fig. 2. In general, the R plasmids showed similar patterns of alteration in cell survival; R2, pMG15, and pPL1 increased UV survival of GMC154 *rec-2*$^+$ *polA3*$^+$ (Fig. 2A) and, to a lesser extent, GMC160 *rec-2*$^+$ *polA3* (Fig. 2C) but failed to alter that of GMC156 *rec-2 polA3*$^+$ (Fig. 2B). The R plasmids also increased the gamma survival of GMC154 (Fig. 2D) but failed to alter that of GMC156 (Fig. 2E) or GMC160 (Fig. 2F).

Spontaneous back-mutation of the *trpB1* and *hisO27* markers. To determine the levels of mutability, two auxotrophic markers, *hisO27* (20) and *trpB1* (formerly *trpF1*; 3), which showed detectable levels of spontaneous back-mutation to *his*$^+$ and *trp*$^+$ were used. Based on their reversion pattern with several mutagens, both auxotrophic markers are assumed to be single base-pair substitution mutations (unpublished data). To obtain a direct comparison of

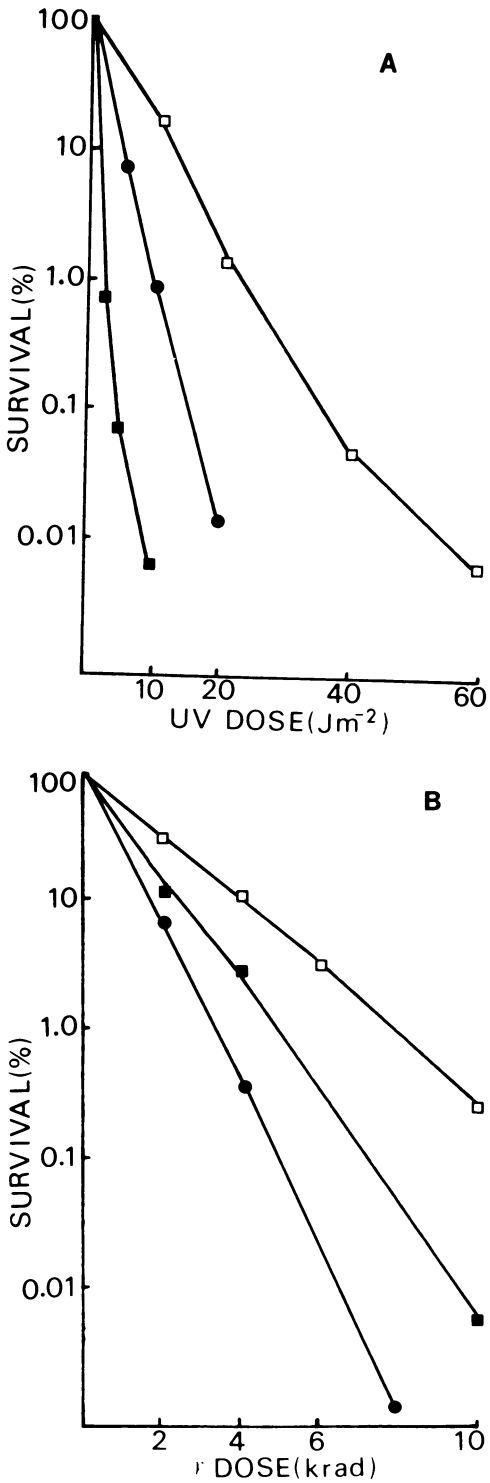

FIG. 1. *Survival of strains after (A) UV irradiation or (B) gamma irradiation. Symbols: □, GMC154; ■, GMC156; ●, GMC160.*

the level of back-mutation to *his*$^+$ and *trp*$^+$, *hisO27* and *trpB1* were combined within a single genetic background (see Materials and Methods). The repair-deficient markers *polA3* (15) and *rec-2* (4) could also be introduced into this genetic background.

The introduction of *rec-2* (GMC156) or *polA3* (GMC160) did not significantly alter the level of spontaneous back-mutation of *hisO27* or *trpB1* (Table 2). In strain GMC154, the presence of R plasmids increased the spontaneous back-mutation to *trp*$^+$ of the *trpB1* marker: 13-fold for pMG15, 5-fold for R2, and 4-fold for pPL1. Back-mutation to *his*$^+$ of *hisO27* was greatly increased by the presence of the R-plasmids (Table 2). The R plasmids were transferred to GMC156, containing the allele *rec-2*, and no increase in spontaneous back-mutation to *trp*$^+$ or *his*$^+$ compared with that of GMC156 was observed. Thus, the ability of these R plasmids to protect host cells against the lethal effects of UV and gamma irradiation and enhance spontaneous mutability is dependent on the *recA*$^+$ genotype.

R plasmid pPL2 (16) is a mutant derivative of pMG2 and was originally selected because it lacked the plasmid-mediated, enhanced, UV-induced mutability of *trpB1*. Strains carrying pPL2 also show an inability to increase the survival of wild-type host cells after UV irradiation (16). Strain GMC154 harboring pPL2 was tested for the level of spontaneous back-mutation to *trp*$^+$ and *his*$^+$. No significant increase in the level of spontaneous back-mutation of *trpB1* or *hisO27* compared with that of GMC154 was observed (Table 2). It therefore appears that a common plasmid-mediated function is involved in alterations of cell survival after UV irradiation and enhancement of spontaneous mutability in *P. aeruginosa*.

In strain GMC160, the presence of R plasmids did not significantly increase the level of spontaneous back-mutation of *trpB1* or *hisO27* (Table 2).

A *pol*$^+$ derivative (GMC164) of GMC160 was obtained as a *trpB1*$^+$ methyl methane sulfonate-resistant recombinant of GMC160 after FP2 conjugation with donor strain PAO170. When R plasmid pPL1 was introduced into GMC164, the level of spontaneous back-mutation of *hisO27* to *his*$^+$ was similar to that of GMC154 containing R plasmid pPL1 (Table 2). Similar results were obtained for R plasmids pMG15 and R2 (unpublished data).

UV-induced and gamma-induced back-mutation of the *trpB1* and *hisO27* markers. Table 3 shows the results of UV-induced and gamma-induced mutation experiments. These data represent typical experiments that were selected from several replicate experiments in

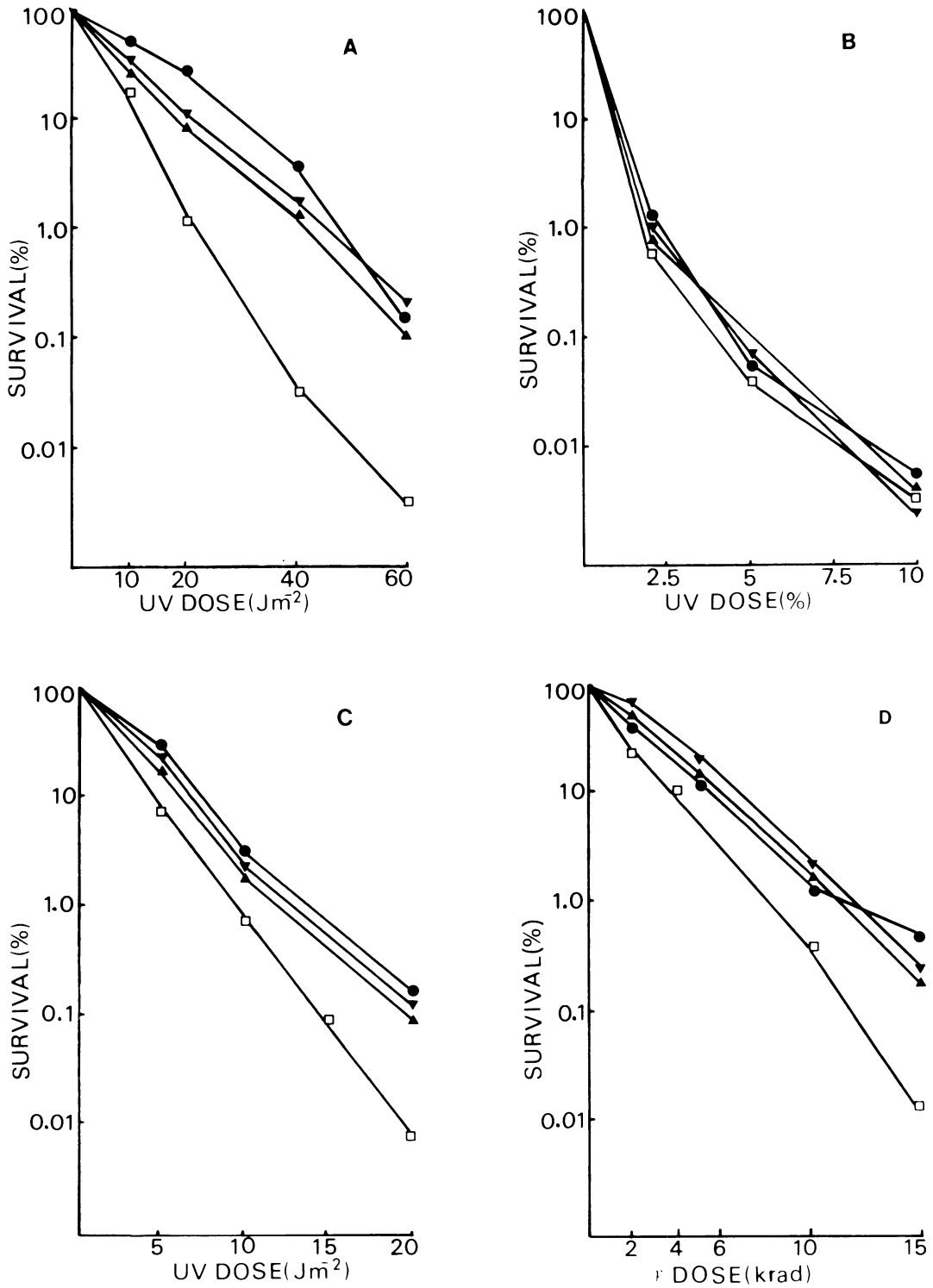

FIG. 2. *Survival after UV irradiation (A, B, C) and gamma irradiation (D, E, F) in strains GMC154 (A, D) GMC156 (B, E), and GMC160 (C, F) without plasmids* (□) *or with plasmids pPL1* (▼), *R2* (●), *and pMG15* (▲).

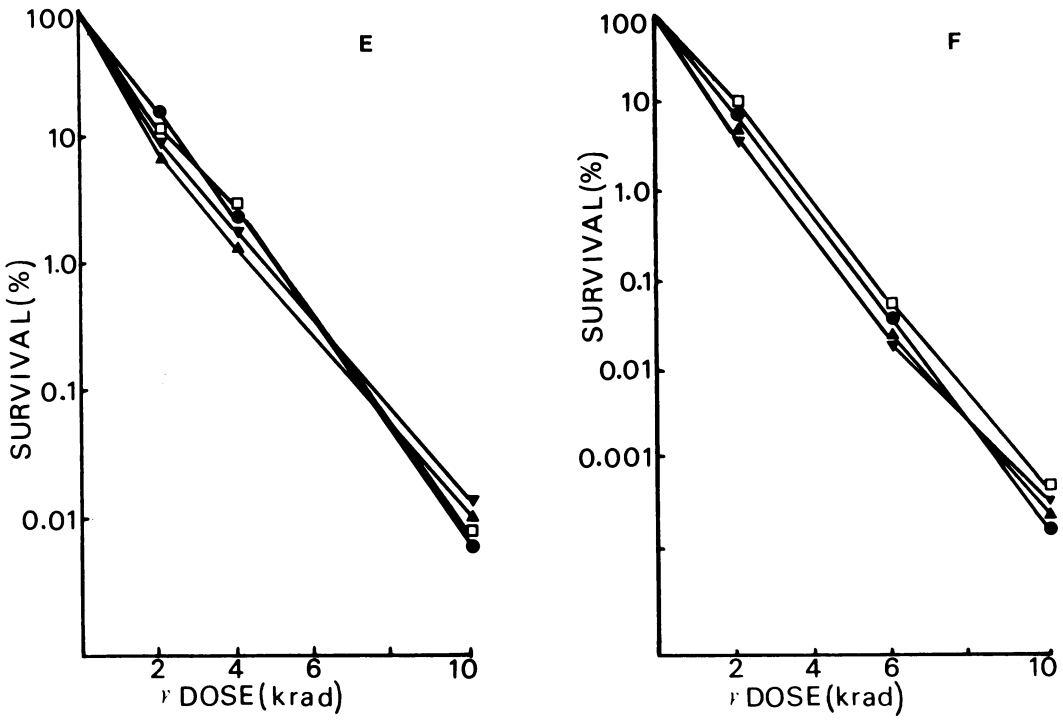

FIG. 2. E-F.

TABLE 2. *Frequencies of spontaneous back-mutation to trp⁺ and his⁺ in strains with and without R plasmids*

Strain	*trpB1* (*trp*⁺ per 10^7 cells plated)[a]	R⁺/R⁻ ratio[b]	*hisO27* (*his*⁺ per 10^7 cells plated)[a]	R⁺/R⁻ ratio[b]
GMC154	1.9 ± 1.1[c]		0.6 ± 0.4	
GMC154(pMG15)	23.9 ± 10.7	12.5	153.3 ± 81.6	255.5
GMC154(pPL1)	6.9 ± 2.8	3.6	59.9 ± 24.4	99.8
GMC154(R2)	9.9 ± 5.9	5.2	34.5 ± 4.5	57.5
GMC154(pPL2)	3.6 ± 1.1	1.9	1.1 ± 0.5	1.8
GMC156	2.7 ± 2.0		0.15 ± 0.2	
GMC156(pMG15)	2.5 ± 1.0	0.9	0.2 ± 0.1	1.3
GMC156(pPL1)	3.0 ± 1.5	1.1	0.3 ± 0.1	2.0
GMC156(R2)	3.6 ± 2.7	1.3	0.3 ± 0.5	2.0
GMC160	3.7 ± 2.0		0.4 ± 0.5	
GMC160(pMG15)	5.0 ± 4.2	1.4	0.5 ± 0.5	1.3
GMC160(pPL1)	9.3 ± 3.6	2.5	1.5 ± 0.9	3.8
GMC160(R2)	5.6 ± 4.2	1.5	0.9 ± 0.6	2.3
GMC164			0.8 ± 0.2	
GMC164(pPL1)			45.6 ± 23.5	57.0

[a] Mean number of back-mutations per 10^7 cells plated.
[b] Frequency of back-mutations per 10^7 cells plated.
[c] Number ± standard deviation.

which the level of killing was no greater than approximately 95%. In strain GMC154 there was a slight increase in the back-mutation of *trpB1* with UV to *trp*⁺, as was previously detected in parental strain *trpB1* (12, 18, 22), whereas the back-mutation of *hisO27* to *his*⁺ was not affected by UV irradiation. The presence of the *polA3* allele (GMC160) increased the UV-induced back-mutation of *trpB1* over the dose range tested, while *hisO27* remained unaffected. No increase in the back-mutation of *trpB1* or *hisO27* was observed in GMC156 (*rec-2*) (data not shown).

In GMC154 the presence of R plasmids substantially increased the UV-induced back-mutation of *trpB1*, while there was a decrease in the number of back-mutations (per plate) of *hisO27* to *his*⁺ with increasing UV doses (Table

TABLE 3. *Frequency of UV- and gamma-induced back-mutation to trp⁺ and his⁺ of strains GMC154 and GMC160 with and without R plasmids*

Strain	UV dose (J/m^2)	Survival (%)	No. of back-mutations per plate		No. of induced back-mutations per 10^7 survivors		Gamma dose (krads)	Survival (%)	No. of back mutations per plate		No. of induced back mutations per 10^7 survivors	
			trp⁺	*his*⁺	*trp*⁺	*his*⁺			*trp*⁺	*his*⁺	*trp*⁺	*his*⁺
GMC154	0	100	13	5			0	100	11	3		
	5	62.5	26	5	5.2		2	30.77	26	6	5	1
	7.5	40	37	5	15		4	10.66	16	6	4.81	2.9
	10	22.5	20	6	7.7		6	1.83	12	4	5.6	5.6
	15	13.8	17	4	7.3							
GMC154(pMG15)	0	100	69	1,036			0	100	138	882		
	5	90.9	418	856	69.8		2	35.6	260	778	34.26	
	7.5	49.1	715	708	239.3		4	11.2	230	978	82.14	85.7
	10	43.6	731	773	275.4		6	4.55	184	1,016	101.0	294.5
	15	23.6	704	603	488.5							
GMC154(pPL1)	0	100	63	544			0	100	64	326		
	5	51.3	592	350	129.0		2	35.14	100	908	18.46	298.5
	7.5	48.8	748	480	175.6		4	13.3	105	784	55.4	618.9
	10	31.8	700	440	250.8		6	5.58	86	840	70.97	1,658.0
	15	16.3	496	326	333.1							
GMC154(R2)	0	100	43	492			0	100	86	274		
	5	63.95	869	360	150.2		2	21.4	272	390	62	38.7
	7.5	57.7	1,140	336	246.5		4	14.3	242	422	78	74
	10	41.9	1,160	319	310.3		6	7.5	182	682	91.4	388.6
	15	34.9	960	303	305.7							
GMC160	0	100	17	3			0	100	12	1		
	1	43.3	57	3	15.4		0.25	31.12	13	3	0.3	0.65
	2	21.0	48	1	24.6		0.5	61.2	13	3	0.16	0.33
	4	7.08	27	2	23.5		1.0	10.2	18	2	6	1
GMC160(pMG15)	0	100	39	8			0	100	39	8		
	1	88.2	204	24	55	5.3	0.25	37.0	29	6		
	2	45.6	188	39	96.1	20	0.5	34.1	31	15		6.03
	4	22.4	222	38	240.8	39.5	1.0	19.05	25	28		30.86
GMC160(pPL1)	0	100	40	17			0	100	26	5		
	1	56.5	128	28	50.3	6.29	0.25	75.0	11	5		
	2	45.2	220	30	128.6	9.3	0.5	45.0	19	4		
	4	26.9	282	31	289.8	16.8	1.0	37.5	11	4		
GMC160(R2)	0	100	47	9			0	100	52	4		
	1	70.3	264	30	48.2	4.6	0.25	36.14	40	5		0.33
	2	52.3	368	39	95.8	8.96	0.5	20.84	47	8		2.3
	4	39.1	340	50	117.2	16.4	1.0	12.0	43	5		1.0

3). In GMC160 the presence of R plasmids produced a similar increase in the level of UV-induced back-mutation of *trpB1* and a slight increase in UV-induced back-mutation of *hisO27*. In GMC156 the R plasmids failed to alter the level of back-mutation of *trpB1* or *hisO27*; no UV-induced back-mutations to *trp*⁺ or *his*⁺ were detected (data not shown).

In GMC154 and GMC160 only a small increase in the number of *trp*⁺ back-mutations per plate was observed after gamma irradiation. This increase was not observed for strain GMC156. GMC154 containing an R plasmid (pPL1, R2, or pMG15) showed an increase in the yield of gamma-induced back-mutations for both *trpB1* and *hisO27*. In the presence of an R plasmid, *trpB1* appears less mutable by gamma irradiation than by UV irradiation, whereas *hisO27* is mutable by gamma irradiation but not UV irradiation. GMC156 (data not shown) or GMC160 containing an R plasmid showed no consistent increase in gamma-induced back-mu-

tation of *trpB1* or *hisO27*. These results indicated that R plasmids pPL1, R2, and pMG15, which are known to alter the survival and mutagenic responses of host cells after UV irradiation (16, 18), also increase wild-type (*polA3*$^+$ *rec-2*$^+$) host cell susceptibility to the mutagenic effects of gamma irradiation. Both enhanced, UV-induced and gamma-induced mutability share a common requirement for a functional *recA*$^+$ gene. However, an analysis of GMC160 containing R plasmids, under conditions which yield substantial levels of UV-induced back-mutations, indicated that R-plasmid-mediated processes leading to gamma-induced mutability and enhanced spontaneous back-mutation may have a common dependence on a functional *polA*$^+$ gene in *P. aeruginosa*.

DISCUSSION

A *P. aeruginosa* strain, containing two revertable auxotrophic markers (*hisO27* and *trpB1*), into which the previously described repair-deficient mutant alleles *polA3* and *rec-2* could be introduced was constructed. The resulting strains (GMC154, GMC156, and GMC160) have enabled us to study the genotypic dependence of spontaneous and radiation-induced mutability, in the presence or absence of R plasmids (pPL1, R2, and pMG15), in this organism.

The mutagenic responses to UV irradiation shown by the *trpB1* marker are similar to those of a variety of auxotrophic markers tested in *E. coli* (25). For *E. coli*, it is considered that the production of mutants after UV irradiation, based on the back-mutation to prototrophy of a sensitive auxotrophic marker, is dependent on the *recA*$^+$ genotype (25) and is the result of an inducible error-prone repair system (25).

In *P. aeruginosa*, it appears that the UV induction of back-mutations to prototrophy is similarly dependent on the *recA*$^+$ genotype, although the presence of an inducible repair system has not been established.

R plasmid R46 (and its derivatives) in *E. coli* (24) and *S. typhimurium* (21) decreases UV sensitivity and enhances UV mutagenesis in the wild type and *uvr* and *polA* mutants but not in *recA* mutant host cells. This suggests that this plasmid exerts its effect by contributing to the error-prone repair system of the cell (21, 23, 24). The results presented for R plasmids pPL1, R2, and pMG15 indicated that there was a similar genotypic dependence for the expression of plasmid-mediated alterations to UV survival and the enhancement of UV-induced mutability in *P. aeruginosa*. The R plasmids also increased the gamma survival and gamma-induced mutability in wild-type host cells, but neither effect was observed in a *polA* or *recA* mutant. Thus, based on the inability of these R plasmids to enhance the level of gamma-induced mutability in a *polA* mutant, a strain in which enhanced UV-induced mutability is detected, a distinction can be drawn between plasmid-mediated processes leading to UV- and gamma-induced mutability in *P. aeruginosa*. This distinction may reflect the operation of a plasmid gene product(s) in differing modes of mutagenesis, having a common requirement for the *recA*$^+$ genotype.

R-plasmid-mediated enhancement of spontaneous and gamma-induced mutability shows a common genotypic dependence for expression. These plasmid effects are not detected in a *polA* or *recA* mutant background. Therefore, spontaneous back-mutation to prototrophy may be the result of plasmid-mediated processes similar to those that act on damage to DNA after gamma irradiation. Previous evidence with the UV-protecting R plasmid R-Utrecht in *S. typhimurium* also indicates a correlation between the enhancement of spontaneous mutability and that of gamma mutability rather than UV mutability (19).

Evidence from *E. coli* (2), based on the isolation of a mutant altered in its mutagenic responses to UV but not gamma irradiation, suggests that different modes of mutagenesis (called constitutive and inducible [1]) are present in excision-proficient (*uvr*$^+$) strains of *E. coli*. Assuming that similar modes of mutagenesis exist in *P. aeruginosa*, then this study indicates that these UV-protecting R plasmids may be capable of enhancing both modes of mutagenesis and that a common plasmid product(s) participates in these *recA*-dependent, error-prone DNA repair pathways.

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