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RESEARCH NOTE

ULTRAVIOLET RADIATION-INDUCED MUTABILITY OF ISOGENIC *uvrA* and *uvrB* STRAINS OF *ESCHERICHIA COLI* K-12 W3110

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INTRODUCTION

In a previous report (Smith, 1976), data were presented showing that an *Escherichia coli* K-12 W3110 *uvrB*5 strain had a higher ultraviolet (UV) radiation-induced reversion frequency than its wild-type parent when plotted on the basis of mutation frequency versus survival. However, for the *E. coli* B/r WP2s *uvrA* strain this higher mutability was observed only at survival levels of 80–100%. This result could be due to genetic background differences between the B/r and K-12 strains, to differences in the auxotrophic marker tested, or to differences in the *uvrA* and *uvrB* mutations. Although both the *uvrA* and *uvrB* mutations result in similar UV sensitivities, the inability to excise pyrimidine dimers, and the lack of the dimer specific UV endonuclease (Howard-Flanders *et al.*, 1966; Braun and Grossman, 1974), there are phenotypic differences between the two mutations; notably, the inviability of *uvrB* but not *uvrA* with *polA*1 (Shizuya and Dykhuizen, 1972; Morimyo and Shimazu, 1976), and the inability of *uvrB* to induce colicin E1 following treatment with UV radiation or mitomycin C (Suzuki and Nakazawa, 1976).

This study was undertaken to determine if the differences in UV mutability reported earlier (Smith, 1976) were due primarily to the *uvrA* and *uvrB* mutations, or to other genetic background differences. To accomplish this, isogenic strains of *E. coli* K-12 W3110 carrying *uvrA*6, *uvrB*5, *uvrA*6 *uvrB*5, and the *uvrA* allele from *E. coli* B/r WP2s were employed.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains employed in this study, their genotypes, and sources or construction are listed in Table 1. The methods of strain construction by transduction have been described (Youngs and Smith, 1973; Smith, 1976).

Media. Cultures were grown in a glucose minimal salts medium (Ganesan and Smith, 1968) supplemented as previously reported (Smith, 1976). Survival and Leu$^+$ reversion were assayed on minimal plates containing either 0.02% wt/v Difco nutrient broth (ΔNB plates) or 1 μg/mℓ L-leucine (Δleu plates). Leu$^+$ revertants arising spontaneously during growth of the cultures were assayed on minimal plates lacking leucine.

Procedure. Log phase cultures were obtained by diluting overnight stationary phase cultures 50-fold into fresh minimal medium, followed by growth at 37°C until a cell density of 1 to 2 × 10^8 cells/mℓ was reached. Cells were collected and washed once in DTM buffer (Ganesan and Smith, 1972) by centrifugation. The washed cells were resuspended at a density of 1 to 2 × 10^8 cells/mℓ in DTM buffer, and UV irradiated. Following UV irradiation, the cells were concentrated 10-fold by centrifugation, and either 0.05 or 0.1 mℓ aliquots were plated directly to assay for Leu$^+$ revertants. Survival was determined by plating 0.1 mℓ aliquots after suitable dilution in 0.067 *M* phosphate buffer pH 7. Plates were scored following incubation at 37°C for 3 days.

Irradiation. The method, source, and dosimetry of UV radiation have been reported (Smith, 1976). The incident

Table 1. Strains of *E. coli* employed

Stanford Strain Number	Source Number	Genotype	Derivation or Source
SR274	B/r WP2s	*uvrA trp*	Smith, 1976
SR114	AB1886	F$^-$ *uvrA*6 *arg pro his leu thr ara gal lac xyl mtl thi str*	Howard Flanders *et al.*, 1966
SR248	KH21	F$^-$ *leuB metE bio rha lacZ malB str thyA thyR*	R. B. Helling
SR250	DY145	F$^-$ *uvrB*5 *leuB metE rha lacZ str thyA thyR*	Youngs and Smith, 1976
SR330	DY246	F$^-$ *uvrB*5 *leuB metE rha lacZ malB str thyA thyR*	P_1 · SR250 × SR248; select Bio$^+$
SR331	DY247	F$^-$ *leuB metE rha lacZ malB str thyA thyR*	
SR312	DY260	F$^-$ *uvrA*6 *leuB metE rha lacZ str thyA thyR*	P_1 · SR114 × SR331; select Mal$^+$
SR314	DY270	F$^-$ *uvrA*6 *uvrB*5 *leuB metE rha lacZ str thyA thyR*	P_1 · SR114 × SR330; select Mal$^+$
SR315	DY271	F$^-$ *uvrB*5 *leuB metE rha lacZ str thyA thyR*	
SR329	DY293	F$^-$ *uvrA leuB metE rha lacZ str thyA thyR*	P_1 · SR274 × SR331; select Mal$^+$

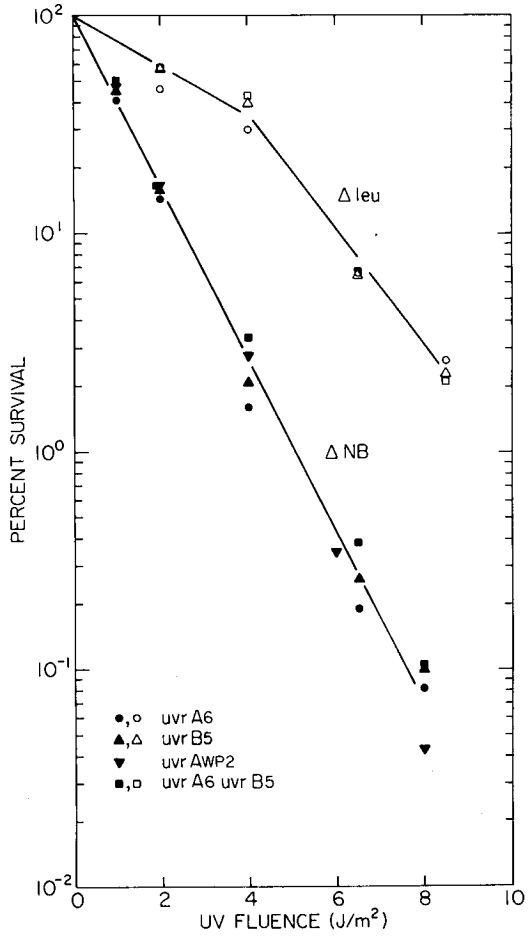

Figure 1. Survival of strains following UV irradiation. Log phase cultures were irradiated in DTM buffer, diluted and plated on either minimal medium supplemented with 0.02% wt/v nutrient broth (ΔNB) or 1 μg/m⁄ L-leucine (Δleu). ●, SR312 *uvrA6* plated on ΔNB; ○, SR312 *uvrA6* plated on Δleu; ■, SR314 *uvrA6 uvrB5* plated on ΔNB; □, SR314 *uvrA6 uvrB5* plated on Δleu; ▲, SR315 *uvrB5* plated on ΔNB; △, SR315 *uvrB5* plated on Δleu; ▼, SR329 *uvrA* WP2s plated on ΔNB. Results on ΔNB plates are the average of four experiments, and the results on Δleu plates are the average of two experiments.

fluence was corrected for sample absorption according to the method of Youngs and Smith (1976).

Calculation of mutation frequency. The formula of Sedgwick and Bridges (1972) was used to calculate the UV-induced Leu$^+$ reversion frequency.

RESULTS AND DISCUSSION

The three strains, SR312 *uvrA6*, *SR*314 *uvrA6 uvrB5* and SR315 *uvrB5* all show similar UV survival on ΔNB and Δleu plates (Fig. 1). Since *E. coli* B/r WP2s carries a *uvrA* mutation (Donch and Greenberg, 1969; Hill, 1970) that may be different from the *uvrA6* allele, we transduced the WP2s *uvrA* mutation into the *E. coli* K-12 W3110 background. Figure 1 also shows that the *uvrA* allele from WP2s results in the same UV survival on ΔNB medium as do the *uvrA6* and *uvrB5* alleles in the W3110 background. Furthermore, Fig. 1 demonstrates that the *uvrA6*, *uvrB5* and *uvrA6 uvrB5* strains show a greater survival on minimal medium than on the more complex ΔNB medium. This increased survival of *uvr*$^-$ strains on minimal medium is known as minimal medium recovery (MMR), and was originally detected using a complex medium, yeast extract nutrient broth (Ganesan and Smith, 1968b, 1972). Our results indicate that only a small amount of nutrient broth is necessary to achieve the sensitizing effect.

Figure 2 shows that there is little difference in the UV-induced Leu$^+$ reversion frequencies of the four *uvr*$^-$ strains tested, when assayed on ΔNB medium. At fluences greater than 4 Jm^{-2}, it appears that the *uvrA6* strain and the strain carrying the *uvrA* allele from WP2s have a somewhat higher mutability than the *uvrB5* and the *uvrA6 uvrB5* strains. However, this small difference is not enough to account for the difference in mutability between the W3110 *uvrB5* strain and the WP2s *uvrA* strain reported by Smith (1976). Since there is little if any difference between the *uvrA6*, *uvrB5* or *uvrA* allele from WP2s on UV survival or Leu$^+$ reversion, the mutational differences reported by Smith (1976) between *uvrB5* W3110 and *uvrA* WP2s and their respective wild-type parent strains are due primarily to the genetic background,

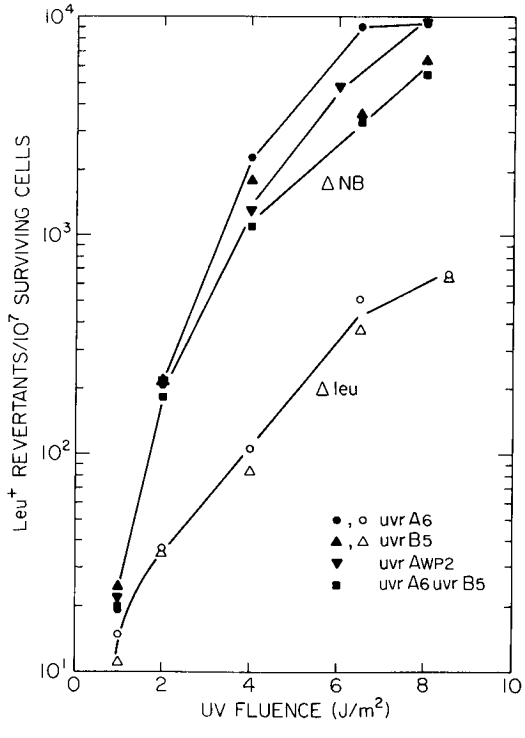

Figure 2. UV-induced Leu$^+$ reversion frequency of strains SR312, SR314, SR315 and SR329 plotted as a function of UV fluence. ●, SR312 *uvrA6* plated on ΔNB; ○, SR312 *uvrA6* plated or Δleu; ■, SR314 *uvrA6 uvrB5* plated on ΔNB; ▲, SR315 *uvrB5* plated on ΔNB; △, SR315 *uvrB5* plated on Δleu; ▼, SR329 *uvrA* WP2s plated on ΔNB. Results on ΔNB plates are the average of four experiments and the results on Δleu plates are the average of two experiments.

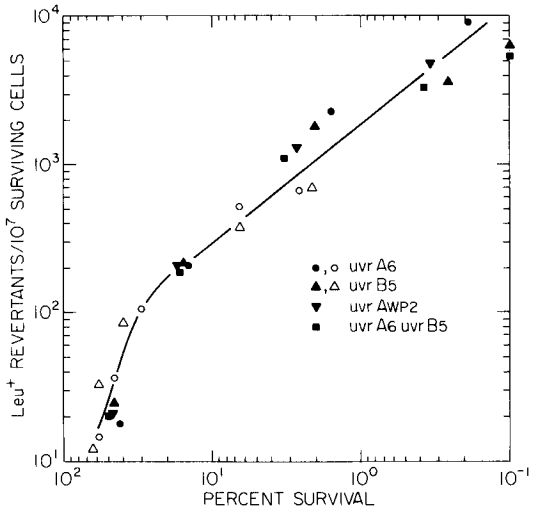

Figure 3. UV-induced Leu$^+$ reversion frequency of strains SR312, SR314, SR315 and SR329 plotted as a function of survival. The data are from the experiments shown in Figs. 1 and 2. The symbols are the same as those for Fig. 2.

and/or the respective auxotrophic marker tested. In this respect the *leuB* marker in the W3110 strains is a missence mutation (R. Bockrath, personal communication) while the tryptophan requirement in WP2s is due to an *ochre* nonsense mutation (Bridges *et al.*, 1967; Osborn and Person, 1967). Therefore, induced mutations at *ochre* suppressing transfer RNA loci would be involved in the reversion of the *trp ochre* marker, but not the *leuB* marker.

Though our data demonstrate that the *uvrA*6 and *uvrB*5 strains have very similar UV-induced reversion frequencies on ΔNB medium, it has been reported that a *uvrA* strain had a much higher UV-induced His$^+$ reversion frequency on Δhis plates than did a *uvrB* strain (Green *et al.*, 1972). To determine if this is true for our *uvrA* and *uvrB* strains, their Leu$^+$ reversion frequencies were assayed on Δleu plates. It can be seen in Fig. 2 that both the *uvrA*6 and the *uvrB*5 strains have the same, but much reduced, Leu$^+$ reversion frequencies on Δleu plates compared to ΔNB plates. This was also true for the double mutant *uvrA*6 *uvrB*5 (data not shown). Therefore, the data of Fig. 2 show that the nutrient broth enrichment of the minimal medium enhanced the Leu$^+$ mutagenesis of both the *uvrA*6 and *uvrB*5 strains at UV fluences greater than $\sim 1\ Jm^{-2}$ (survival: $< 50\%$). A smaller broth enhancement of UV-induced reversion has also been reported for *E. coli* WP2s *uvrA* (Munson and Bridges, 1966; Clarke, 1967). However, when the Leu$^+$ reversion frequencies obtained on ΔNB and Δleu media are plotted as a function of survival (Fig. 3), there is no apparent difference in reversion frequency between the two media; indicating that broth enrichment does not specifically enhance mutagenesis over lethality in our *uvr*$^-$ strains. Furthermore, the dramatic change in slope seen in Fig. 3 for the production of mutations at survival levels below about 30–40% may suggest different molecular mechanisms for mutagenesis over these two ranges of cell survival.

Our data indicate that the enrichment of minimal medium with a small amount of nutrient broth (ΔNB) is sufficient to inhibit MMR and to enhance Leu$^+$ reversion of the *leuB* missense mutation in our *uvr*$^-$ strains. This suggests that there may be a relationship between MMR and error-free postreplication repair. Further research is in progress to clarify the relationship between MMR and broth enhancement of UV-induced mutagenesis in *uvr*$^-$ strains of *E. coli* K-12 W3110.

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REFERENCES

Braun, A. and L. Grossman (1974) *Proc. Natl. Acad. Sci. U.S.* **71**, 1838–1842.
Bridges, B. A., R. E. Dennis and R. J. Munson (1967) *Genetics* **57**, 897–908.
Clarke, C. H. (1967) *Mol. Gen. Genet.* **99**, 97–108.
Donch, J. and J. Greenberg (1969) *Mutat. Res.* **8**, 658.
Ganesan, A. K. and K. C. Smith (1968a) *J. Bacteriol.* **96**, 365–373.
Ganesan, A. K. and K. C. Smith (1968b) *Cold Spring Harbor Symp. Quant. Biol.* **33**, 235–242.
Ganesan, A. K. and K. C. Smith (1970) *J. Bacteriol.* **102**, 404–410.
Ganesan, A. K. and K. C. Smith (1972) *J. Bacteriol.* **111**, 575–585.
Green, M. H. L., M. A. Rothwell and B. A. Bridges (1972) *Mutat. Res.* **16**, 225–234.
Hill, R. F. (1970) *Mutat. Res.* **9**, 341–344.
Howard-Flanders, P., R. P. Boyce and L. Theriot (1966) *Genetics* **53**, 1119–1136.
Morimyo, M. and Y. Shimazu (1976) *Mol. Gen. Genet.* **147**, 243–250.
Munson, R. J. and B. A. Bridges (1966) *Mutat. Res.* **3**, 461–469.
Osborn, M. and S. Person (1967) *Mutat. Res.* **4**, 504–507.
Sedgwick, G. S. and B. A. Bridges (1972) *Mol. Gen. Genet.* **119**, 93–102.
Shizuya, H. and D. Dykhuizen (1972) *J. Bacteriol.* **112**, 676–681.
Smith, K. C. (1976) *Photochem. Photobiol.* **24**, 433–437.
Suzuki, N. and A. Nakazawa (1976) *Nature* **261**, 244–245.
Youngs, D. A. and K. C. Smith (1973) *J. Bacteriol.* **116**, 175–182.
Youngs, D. A. and K. C. Smith (1976) *J. Bacteriol.* **125**, 102–110.