Far Ultraviolet Light Sensitive Derivatives of *Streptomyces clavuligerus*

By G. SAUNDERS* AND G. HOLT

School of Engineering and Science, The Polytechnic of Central London, 115 New Cavendish Street, London W1M 8JS, U.K.

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Five far ultraviolet light sensitive (Uvs) strains – CL77, CL89, CL90, CL104 and CL105 – were isolated from an auxotrophic strain of *Streptomyces clavuligerus* (ATCC 27064). When compared to their progenitor, strain CL7, all exhibited a changed mutagenic response to far ultraviolet light (FUV). Strains CL77 and CL104 failed to yield detectable mutants, whereas induced mutants were obtained in strains CL90 and CL89, but only at low FUV doses (up to 20 J m^{-2}). Strain CL89 was hypermutable at these low doses relative to strain CL7. Strain CL105 gave an increased response in terms of mutants per surviving cell. Caffeine increased the FUV-induced mutation frequency at a particular FUV dose in strain CL7 by up to 3000%. All the Uvs strains showed an altered response to caffeine compared with strain CL7.

INTRODUCTION

DNA repair may be considered as an obligate step in the process of 'indirect' mutation induction intercalated between production of a pre-mutational lesion and realization of a mutant clone. By interfering with DNA repair, either by chemical inhibition of repair enzymes or mutation of DNA repair genes, one may in turn affect the process of mutation induction.

The first radiation-sensitive (i.e. genetically repair-deficient) mutant was isolated by Hill (1958) from *Escherichia coli*. Since then the number of such mutants has steadily grown. In *E. coli*, for example, there are at present some 25 loci known to be involved in DNA repair of damage induced by far ultraviolet light (FUV), and in all of these the mutagenic response to FUV, and in certain cases to other mutagens, is altered (Kimball, 1978).

Kondo *et al.* (1970) have studied the response of FUV-sensitive (Uvs) strains of *E. coli* to different mutagens. They found that *rec* strains, which failed to yield mutants in response to FUV and ionizing radiations, were mutated by the alkylating agent *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG). However, methyl methanesulphonate (MMS), another alkylating agent, followed the pattern of FUV and ionizing radiations in that no MMS-induced mutants were obtained in *rec* strains. Previously, Witkin (1967) had reported that *exr* strains were mutated normally by NTG.

The secondary metabolites of actinomycetes provide the majority of industrially and agriculturally important antibiotics (Bérdy, 1974). Strains used industrially will usually be subjected to a programme of strain selection and improvement, in which a clear insight into the mechanism of mutation induction in the particular strain under investigation would be useful.

Streptomyces clavuligerus has been shown to produce a new antibacterial agent, clavulanic acid (Reading & Cole, 1977), and in the present study we have tested the response of Uvs derivatives of *S. clavuligerus* to different mutagens including FUV, ionizing radiations and alkylating agents. Additionally, the effect of caffeine on FUV-induced mutation frequencies has been investigated in both repair-deficient and repair-proficient strains.

METHODS

Media. Yeast extract agar (YEA) and purified agar (PA) were from Oxoid. Yeast extract-malt extract agar (YM) was as described in Shirling & Gottlieb (1966). All media were prepared at pH 7.2.

Organism and cultural conditions. *Streptomyces clavuligerus* 27064N, a subculture of strain ATCC 27064, was obtained from Beecham Pharmaceuticals and maintained on YM.

Isolation of Uvs strains. A spore suspension of a hypoxanthine-requiring auxotroph of *S. clavuligerus* 27064N was irradiated with FUV to give approximately 20% survival (48 J m^{-2}). Survivors were spread on YEA plates and examined for FUV sensitivity using the technique devised by Harold & Hopwood (1970).

Preparation of spore suspensions. Spores were harvested from 14–21 d old cultures grown at 26°C on YM slopes and resuspended in 0.01% (v/v) Tween 80 (Hopkin & Williams) by mixing on a vortex blender together with glass beads. Suspensions were then filtered through sintered glass (Fisons, porosity 1). Spore suspensions were prepared to give approximately 4×10^8 spores ml^{-1} .

Mutagenic treatments. A Hanovia bactericidal FUV lamp giving 95% of its output at 254 nm was used. Dose was measured using a 'Blak-ray' meter (Ultra-violet Products, San Gabriel, Calif., U.S.A.). Spore suspensions were irradiated in 1.5 ml quantities in glass Petri dishes of 3 cm diameter. During irradiation the spore suspension was agitated by a magnetic stirrer.

A Siemens Dermoplan therapy unit generating X-rays of 50 kV (250 mA), with the beam directed vertically into an open Universal bottle containing 0.5 ml of sample, was also employed. The calculated dose rate was 537 rad min^{-1} .

Methyl methanesulphonate (MMS) treatment was carried out in phosphate buffer pH 7. Spores suspended in Tween 80 were sedimented and resuspended in buffer. MMS was added and after 15 min spores were once more sedimented, washed, and then resuspended in Tween 80. MMS concentrations used ranged from 0.05 to 0.2% (v/v). *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) treatment was carried out in the same way except that citrate buffer, pH 5.6, was used. NTG concentrations used ranged from 0.5 to 3.0 mg ml^{-1} .

Assay for mutagenesis. After mutagenic treatment, 0.1 ml samples of undiluted spore suspension were mixed with 10 ml of soft YEA (0.8% agar) (layer 1) and poured into a Petri dish. After allowing time for the agar to set, a second 10 ml layer (layer 2) of soft agar was poured on top. Plates were then incubated for 20 h at 26°C , a time selected after experimentation as the optimum time to obtain expression. At the end of this period, a third (layer 3) 10 ml layer of PA containing either acriflavine or rifampicin was added. The final concentrations of acriflavine and rifampicin in a plate were $50 \mu\text{g ml}^{-1}$ and $1 \mu\text{g ml}^{-1}$, respectively. Plates were incubated at 26°C for 7 d and then scored for mutants. Survival plates were prepared in a similar manner containing a final volume of 30 ml but no selective agent.

Caffeine, when used, was added to layer 1, prior to pouring, at a final concentration of 1 mg ml^{-1} . Solutions of caffeine were made up in distilled water and membrane filtered (pore size $0.22 \mu\text{m}$).

RESULTS

The relative sensitivities to FUV of five Uvs strains (CL77, CL89, CL90, CL104 and CL105) compared to strain CL7, the hypoxanthine-requiring auxotroph of *S. clavuligerus* from which they were derived, are illustrated in Fig. 1.

Survival after FUV treatment of strain CL7 and the FUV-sensitive derivatives in the presence and absence of caffeine is shown in Table 1. Caffeine (1 mg ml^{-1}) had previously been shown to have no toxic effect on untreated spores. It can be seen that the presence of caffeine also had no significant effect on the survival after FUV treatment of strains CL89, CL90 and CL105. However, caffeine enhanced the killing effect of FUV in strains CL7, CL77 and CL104.

The mutagenic responses of strains CL7, CL89, CL90 and CL105 in the presence and absence of caffeine are shown in Table 2. Caffeine increased the frequency of FUV-induced mutation to rifampicin resistance in strains CL7, CL90 and CL105. However, it had no effect on the induced mutation frequency in strain CL89 and the magnitude of the effect was significantly lower in strain CL105 than in CL7. Mutants CL89 and CL105 were hypermutable relative to strain CL7.

In strain CL90, mutants were only obtained at very low FUV doses (up to 20 J m^{-2}). The mutagenic response of strain CL90 to X-rays was similar to that of strain CL7, whereas, compared to strain CL7, strain CL90 was only slightly mutated by MMS. For example, an MMS dose giving 170 acriflavine-resistant mutants per 10^7 survivors in strain CL7 gave only 10 per 10^7 survivors in strain CL90.

Fig. 1. FUV survival curves of the progenitor strain CL7 (○) and Uvs strains CL77 (●), CL105 (□), CL104 (■), CL90 (△) and CL89 (▲).

Table 1. *Percentage survival after FUV treatment of the wild-type strain (CL7) and FUV-sensitive derivatives, grown in the absence and presence of caffeine*

Strain	Percentage survival									
	Caffeine absent					Caffeine present				
	FUV dose (J m^{-2}):					FUV dose (J m^{-2}):				
	0	5	10	15	20	0	5	10	15	20
CL7	100	97.2	98.0	92.9	95.5	100	79.7	81.3	78.5	50.1
CL77	100	95.6	90.2	84.7	80.3	100	90.2	81.7	69.2	55.6
CL89	100	73.0	44.6	10.0	4.7	100	68.7	39.5	11.0	5.3
CL90	100	74.7	51.6	35.2	24.4	100	77.1	56.3	29.2	24.8
CL104	100	80.9	69.6	44.7	16.4	100	44.2	13.2	4.1	2.0
CL105	100	85.1	67.0	43.1	34.3	100	87.3	67.0	37.1	28.1

Table 2. *Frequency of FUV-induced mutation to rifampicin resistance for the wild-type strain (CL7) and FUV-sensitive derivatives, grown in the absence and presence of caffeine*

Strain	Mutants per 10^7 survivors									
	Caffeine absent					Caffeine present				
	FUV dose (J m^{-2}):					FUV dose (J m^{-2}):				
	0	5	10	15	20	0	5	10	15	20
CL7	0.8	2.9	6.1	14.0	18.0	0.9	91.0	166.0	198.0	212.0
CL89	0.5	74.0	118.0	43.0	50.0	0.4	50.0	95.0	54.0	32.0
CL90	0.5	0.20	4.4	9.3	16.0	0.3	10.4	13.2	34.4	35.2
CL105	1.6	18.0	94.0	124.0	184.0	2.5	72.0	200.0	400.0	820.0

In all experiments performed with strains CL77 or CL104 no detectable FUV-induced mutants were obtained. Both strains showed significantly lower mutation frequencies than strain CL7 when treated with the alkylating agent MMS. For example, an MMS dose giving

255 acriflavine-resistant mutants per 10^7 survivors in strain CL7 gave only 71 in strain CL77 and 56 in strain CL104.

Mutants CL89, CL105 and CL77 showed a similar mutagenic response to NTG to that observed in the wild-type strain CL7 (isolates CL90 and CL104 were not tested for their response to NTG). All strains tested gave similar dose response curves with NTG treatment, reaching a maximum mutation frequency of between 130 and 220 rifampicin-resistant mutants per 10^7 survivors.

DISCUSSION

Caffeine has been shown to inhibit excision repair in *E. coli* (Sideropoulos & Shankel, 1968) and, prior to this, Lieb (1961) demonstrated that FUV-induced mutation frequencies in bacteria could be increased by caffeine. In our study, caffeine similarly affected mutation frequencies in wild-type *S. clavuligerus*. The reduced effect of caffeine in mutant CL105 and its apparent total ineffectiveness in mutant CL89 suggests that both these Uvs mutants are excision-deficient, but possibly affected in different components of the excision repair system.

Hypermutability is a characteristic of excision-deficient strains of *E. coli* (Witkin, 1975). The type of hypermutability observed in strain CL89 (where mutation frequency reaches a peak and then declines) is identical to a measure of SOS activity in *E. coli polA* mutants (Witkin, 1976). The gene product of *polA* is DNA polymerase I thought to effect the excision and resynthesis steps of excision repair in *E. coli*. On the other hand, the pattern of hypermutability observed in strain CL105 is similar to that found in *E. coli exc* mutants deficient in the initial nicking or incision step of excision repair (Kondo *et al.*, 1970).

Fong & Bockrath (1979) suggested that caffeine inhibits the incision step of excision repair in *E. coli*. The insensitivity of strain CL89 to caffeine is consistent with the suggestion that CL89 is polymerase-deficient, and thus the effect of inhibiting the incision step in this strain is negated by the absence of the next step, excision and/or resynthesis. Accordingly, strain CL105 could be deficient in the initial incision step of excision repair and consequently exhibit a reduced sensitivity to caffeine and an increased mutability towards FUV.

The phenotype of strain CL77 is such that it can readily be compared to *lex* mutants of *E. coli* (Witkin, 1967). Strain CL77 and *lex* mutants have the common characteristic of yielding no detectable mutants when treated with FUV and X-rays and significantly reduced mutation frequencies when treated with MMS. Additionally, they are both mutated normally by NTG. Strain CL104 was also similar in these respects, but it was far more sensitive to killing by FUV than strain CL77 and for this reason we suggest that strain CL104 could carry a mutation of the *recA* type found in *E. coli*. Thus, we suggest that strains CL77 and CL104 carry mutations at loci necessary for the operation of an error-prone pathway concerned with mutation induction by FUV, X-rays and MMS and that NTG produces mutations through a distinct pathway.

Strain CL90, whilst exhibiting a normal response to X-rays, showed a marked change in its response to FUV and MMS. Unless one supposes that caffeine can increase the mutation frequency in more than one way, which seems unlikely bearing in mind the results obtained with strains CL89 and CL105, the potentiation of FUV-induced mutation frequency observed in this strain at low doses implies that the excision system for FUV damage is operating. Further support for this is provided by the observation that the suggested excision-deficient strains CL89 and CL105 are normal in their mutagenic response to MMS, whilst CL90 is not. We therefore suggest that mutation induction by X-rays and FUV have only some steps in common.

Harold & Hopwood (1970) isolated some 20 Uvs strains of *Streptomyces coelicolor* but provided no evidence as to whether the mutagenic response of these strains differed from that of the wild-type. Results obtained in our laboratory (Coleman & Holt, see Saunders *et al.*, 1982) have confirmed that in *S. coelicolor* certain *Uvs* loci also confer hypermutability on a strain.

The ubiquity of DNA repair mechanisms in living organisms from bacteria to man (Cleaver, 1968) is well known, and our studies indicate that *S. clavuligerus* has such repair mechanisms and that their relationship to mutation induction appears to parallel closely the relationships observed in *E. coli*.

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