Absence of Error-prone Repair in a *Vibrio* species

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The effect of various DNA-damaging agents on a *Vibrio* species was investigated. The organism was readily mutable by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and mitomycin C but not by UV light. No Weigle reactivation of UV-irradiated α 3a phage was detected. These results suggest that an error-prone repair mechanism is lacking in this species.

INTRODUCTION

Vibrio sp. JT2 is a halotolerant collagenolytic Gram-negative bacterium which was isolated from cured hides and is associated with leather decay (Thomson *et al.*, 1972). The strain is an interesting one in that it carries a cryptic prophage, α 3a, inducible by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and mitomycin C (MC) but not by UV irradiation. It has been proposed that a defective enzyme necessary for prophage excision and/or vegetative phage development, produced by the cryptic prophage, can be reverted or suppressed by mutagens and lead to prophage induction (Thomson & Woods, 1974). Such a proposal would be supported by the finding that whereas MNNG and MC are able to induce mutations in *Vibrio* JT2, UV irradiation is not.

Mutations resulting from treatment with UV (Drake & Baltz, 1976; Walker & Dobson, 1979) are caused by the induction of a highly error-prone repair system, one of the responses of the so-called SOS system (Miller, 1983; Radman, 1975; Walker, 1984; Witkin, 1976). MNNG is a mutagen whose action is due to alkylation of DNA largely independent of the *recA* gene product in *Escherichia coli* (Gichner & Veleminsky, 1982; Iishi & Kondo, 1975) and thus organisms lacking the SOS repair system are readily MNNG mutable. MC is thought to cause mutations by the repair of MC-induced cross-linkages in DNA, although the exact mechanism appears to be unclear (Boyce & Howard-Flanders, 1964). The present study was aimed at investigating the mutagenic actions of MNNG, MC and UV light on *Vibrio* JT2. As UV reactivation of UV-irradiated phage (Weigle reactivation; Weigle, 1953) depends on the induction of an active error-prone repair system (Witkin, 1976), this process was also investigated in *Vibrio* JT2.

According to the proposal outlined above the presence or absence of the cryptic prophage should have no effect on the mutagenic action of these three agents. To verify this, a derivative of JT2, JTc39, which had been cured of its prophage, was used as a control.

METHODS

Bacterial and phage strains. The *Vibrio* strains used were all derivatives of *Vibrio* sp. JT2, a prototrophic, halotolerant, collagenolytic bacterium isolated from cured hides (Thomson *et al.*, 1972, 1981; Robb *et al.*, 1980). The auxotrophic mutants for use in reversion studies were JT-21 Trp⁻ and JT-28 Ile⁻, which were isolated by

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Abbreviations: MC, mitomycin C; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

Fig. 1. Sensitivities of strains to (a) UV irradiation, (b) MNNG and (c) MC treatment as described in the text. Data represent the means of three experiments. ●, *E. coli* C600; ■, *Vibrio* JT2; ▲, *Vibrio* JTc39.

MNNG mutagenesis of JT2. Strain JTc39 is a derivative of JT2 cured of its cryptic $\alpha 3a$ prophage by treatment with MNNG followed by colony hybridization with ^{32}P -labelled $\alpha 3a$ phage DNA (unpublished results). *Escherichia coli* C600 was used for comparative purposes. The bacteriophage was $\alpha 3a$ isolated after spontaneous liberation by JT2 (Thomson & Woods, 1974).

Media and chemicals. Specific media for *Vibrio* JT2 were used and Luria broth was supplemented with 0.4 M-NaCl (LSB; Thomson & Woods, 1974; Woods & Thomson, 1975). All amino acids and MC were obtained from Sigma, and MNNG from Koch-Light.

UV, MNNG and MC treatment. For survival determination, exponentially growing cultures were washed and resuspended in 0.4 M-NaCl (*Vibrio*) or 0.145 M-NaCl (*E. coli*). Samples to be tested for UV sensitivity were irradiated in 10 ml portions in a 100 mm glass Petri dish with constant stirring at increasing doses using a model UVS-54 Mineralight shortwave UV 254 nm lamp. Appropriate dilutions were plated immediately. All manipulations were carried out in the dark to eliminate photoreactivation. UV doses were measured using a Blak-ray UV meter model J-225 (Ultra-violet Products, Inc.). Samples of *Vibrio* to be tested for MNNG and MC sensitivity were resuspended in LSB with increasing concentrations of the DNA-damaging agent and incubated at 37 °C for 15 min prior to plating. For the isolation of mutants, survivors were plated either on LSB agar containing rifampicin ($3 \mu\text{g ml}^{-1}$) or nalidixic acid ($0.2 \mu\text{g ml}^{-1}$) or, in the case of reversion of JT-21 and JT-28 to prototrophy, on minimal medium.

Weigle reactivation of $\alpha 3a$ phage. Phage $\alpha 3a$, prepared as before (Thomson & Woods, 1974) was UV irradiated and plated on *Vibrio* JT-21 cells irradiated with increasing doses of UV light according to the method of Weigle (1953). Phage titres were determined as before (Thomson & Woods, 1974).

RESULTS

Sensitivity of *Vibrio* to UV, MNNG and MC

Exponentially growing *Vibrio* JT2 and JTc39 were both more resistant to UV irradiation than repair-proficient *E. coli* 600 (Fig. 1a). Thus, if this *Vibrio* species lacks an error-prone repair

Fig. 2. Mutagenesis of *Vibrio* JT2 and JTc39 by MNNG and MC. Exponential-phase cells were incubated in medium containing increasing concentrations of MNNG or MC as described in the text. Data represent the means of three experiments. (a) MNNG mutagenesis. ●, ○, JT2 resistant to nalidixic acid (●, 0.2 $\mu\text{g ml}^{-1}$; ○, 2 $\mu\text{g ml}^{-1}$); ■, □, JTc39 resistant to nalidixic acid (■, 0.2 $\mu\text{g ml}^{-1}$; □, 2 $\mu\text{g ml}^{-1}$); ▲, JT2 resistant to rifampicin (3 $\mu\text{g ml}^{-1}$). (b) MC mutagenesis. ●, ○, JT2 resistant to nalidixic acid (●, 0.2 $\mu\text{g ml}^{-1}$; ○, 2 $\mu\text{g ml}^{-1}$); ■, JTc39 resistant to nalidixic acid (0.2 $\mu\text{g ml}^{-1}$); ▲, △, JT2 resistant to rifampicin (▲, 3 $\mu\text{g ml}^{-1}$; △, 1.5 $\mu\text{g ml}^{-1}$).

system, it must have other efficient UV repair systems. The sensitivity of JT2 and JTc39 to MNNG and MC were investigated in order to determine the conditions to be used for mutagenesis studies (Fig. 1*b, c*).

Mutability of *Vibrio*

Exposure of prototrophic JT2 and JTc39 to MNNG and MC resulted in the isolation of Rif^R and Nal^R mutants (Fig. 2), while treatment of JT-21 Trp⁻ and JT-28 Ile⁻ yielded prototrophic revertants (Table 1). The optimal MNNG concentration for the isolation of mutants of JTc39 resistant to 0.2 μg nalidixic acid ml^{-1} was 2–5 $\mu\text{g ml}^{-1}$, which corresponded to a survival frequency of about 35%, whereas that for JT2 (at least 10 $\mu\text{g ml}^{-1}$) corresponded to a survival frequency of less than 10%. Very few mutants of either strain resistant to 2 μg nalidixic acid ml^{-1} were isolated. Whereas Rif^R mutants of JT2 were readily isolated, no Rif^R mutants of JTc39 were found (Fig 2*a*). When MC was used as a mutagen (Fig. 2*b*), JT2 mutants resistant to 0.2 μg nalidixic acid ml^{-1} , and to a lesser extent to 2 $\mu\text{g ml}^{-1}$, were isolated as well as mutants resistant to 1.5 and 3 μg rifampicin ml^{-1} , but the mutation frequency was an order of magnitude lower than for MNNG. Nal^R mutants of JTc39 were also isolated at low frequencies. Revertants of both JT-21 and JT-28 to Trp⁺ and Ile⁺ respectively were isolated after treatment with MNNG although it was apparent that the *trp* mutation reverted at a considerably greater frequency than *ile* (Table 1). Reversion by MC was not investigated.

Table 1. *MNNG-induced reversion of auxotrophic mutants*

Data are the means of three experiments. Frequencies shown as <0.1 represent examples where no revertants were isolated.

Percentage survival	No. of Trp ⁺ revertants of JT-21 per 10 ⁸ c.f.u. after		No. of Ile ⁺ revertants of JT-28 per 10 ⁸ c.f.u. after	
	72 h	120 h	72 h	120 h
100	<0.1	0.1	<0.1	<0.1
24	21	25	0.6	0.8
1	520	55	13	25
0.1	141	150	1.3	2

Table 2. *UV mutagenesis of Vibrio strains*

Data are the means of three experiments. Plates were counted after 48 and 72 h but were checked again after 120 h with no change in the mutation frequencies.

Strain	Percentage survival	No. of JT2 Rif ^R mutants per 10 ⁷ c.f.u.	No. of JT2 Nal ^R mutants per 10 ⁷ c.f.u.	No. of JT-21 Trp ⁺ revertants per 10 ⁷ c.f.u.	No. of JT-28 Ile ⁺ revertants per 10 ⁷ c.f.u.
JT2, JT-21 and JT-28	100	0.4	0.4	0.1	<0.1
	63	<0.4	ND	0.1	ND
	50	<0.5	0.5	ND	0.1
	10	0.1	0.6	<0.5	0.1
	3	ND	<0.3	ND	<0.3
	0.1	<0.5	<0.4	0.1	ND
JTc39	100	<0.4	<0.5		
	61	<0.4	<0.4		
	0.1	<0.3	<0.3		

ND, Not done.

Table 3. *Growth of UV irradiated phage α3a in UV-treated JT2*

Data are the means of three experiments. There was no significant difference in results between incubation temperatures of 30 °C and 37 °C.

Percentage survival of JT2	Time of post-irradiation incubation of JT2 (h)	Relative survival of α3a*
100	0	0.40
	2	0.40
	4	0.45
	6	0.50
95	0	0.40
	2	0.40
	4	0.46
	6	0.30
90	0	0.30
	2	0.35
	4	0.30
	6	0.28
70	0	0.35
	2	0.35
	4	0.40
	6	0.35

* Survival frequencies are given relative to the p.f.u. of unirradiated α3a plated on unirradiated JT2 without post-irradiation incubation.

Strains JT2, JT-21, JT-28 and JTc39 were exposed to increasing doses of UV radiation to give decreasing survival frequencies, but no increase in the number of mutants isolated was found for any of the four markers tested (Table 2). This was also the case when sublethal concentrations of caffeine (1 and 2 mg ml⁻¹), a known inhibitor of excision repair in prokaryotes (Timson, 1977), was included in the selective media (data not shown). [In *E. coli*, UV-induced mutagenesis increases in excision repair deficient mutants, but as no such mutants exist in this *Vibrio* species caffeine was used instead.]

Weigle reactivation

To determine whether UV-irradiated phage α 3a could be reactivated by a UV-inducible repair system in JT2, the phage was irradiated to give a survival frequency when plated on unirradiated JT2 of 50%. Exponentially growing cells of JT2 were irradiated with low doses of UV light to give survival frequencies of 70–98%. Irradiated cells were washed and incubated in LSB for 1–5 h at 30 °C and 37 °C to allow for possible induction of repair proteins. No reactivation of irradiated phage α 3a was found under any of the conditions used (Table 3).

DISCUSSION

Both *Vibrio* JT2 and its cured derivative JTc39 were more resistant to UV-irradiation than repair-proficient *E. coli* C600. This is in contrast to most bacteria that lack a form of post-replicative repair, which are generally more sensitive to UV-induced lethality.

Both JT2 and JTc39 were readily mutated by MNNG and MC for the markers selected, although in general JTc39 yielded fewer mutants. The significance of this has not yet been determined. As expected, MNNG was a far more potent mutagen than MC. Conversely no increase above the spontaneous mutation frequency was found after UV-irradiation of JT2 or JTc39. That this reflects the absence in these strains of a UV-inducible error-prone post-replicative repair system was supported by the absence of UV-inducible phage reactivation (Weigle reactivation).

It has been proposed that since many bacterial species which lack error-prone repair are competent for DNA-mediated transformation, competency is not compatible with error-prone repair (Sicard, 1983; Yasbin, 1977). This correlation has been questioned, as *Proteus mirabilis* and *Salmonella typhimurium* appear to be deficient in this repair system (Hofemeister *et al.*, 1979; Walker, 1978). However, both these species possess at least some elements of the SOS response (Walker, 1984). It would therefore be of interest to investigate whether this *Vibrio* species has any SOS-like responses.

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