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Induction and Characterization of pKM101 Mutants in *Salmonella typhimurium*

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*Salmonella typhimurium*內로의 pKM101 돌연변이체의 유도와 그 특성에 관한 연구

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ABSTRACT

Mutants of plasmid pKM101 modified to enhance mutagenesis were induced and characterized in *Salmonella typhimurium*.

The pKM101 mutant plasmid were transferred normally and stably maintained in cells. They had modified in their ability (i) to enhance the reversion of both point and frameshift mutations, (ii) to protect the cell against UV-irradiation and chemical mutagen treatment, (iii) of ampicillin resistance. A similar modification in enhancement of reversion was also observed in a *uvrB*$^-$ strains. These results indicated that mutator effect of pKM101 was coded by one plasmid gene.

INTRODUCTION

pKM101 is transmissible extrachromosomal genetic elements capable of conferring resistance to ampicillin on their hosts. This plasmid has been shown to enhance the reversion of both point and frameshift mutation by a variety of carcinogens and mutagens in the *Salmonella* tester system (McCann *et al.*, 1976). The drug resistance pKM101 is one of several which has been found to reduce the lethal effect of UV-irradiation and enhance the mutagenic effect of UV in respect to the reversion of some auxotrophic mutants (Drabble *et al.*, 1968).

In addition to the enhanced spontaneous reversion, pKM101 (i) is of the N compatibility, (ii) is self-transmissible, (iii) is *fi*$^-$, (iv) lack a type II restriction system, (v) codes for a new ATP independent single and double srand endonuclease, (vi) enhance reactivation of UV-irradiated phage in both unirradiated *Salmonella typhimurium* LT2 and *Esherichia coli* K12

Table 1. Properties of bacterial strains used

Strain No.	Genotype chrom/plasmid	Source
G46	*his* (base substitution)	B.N. Ames
D3052	*his* (frameshift)	B.N. Ames
TA1535	*his*G46 *uvr*B *rfa*	B.N. Amese
TA1538	*his*D3052 *uvr*B *rfa*	B.N. Ames
TA100	*his*G46 *uvr*B *rfa*/pKM101	B.N. Ames
TA98	*his*D3052 *uvr*B *rfa*/pKM101	B.N. Ames
TA1975	*his*G46 *rfa*	B.N. Ames
TA1978	*his*D3052 *rfa*	B.N. Ames
TA1975/pKM101	TA1975/pKM101	TA1975/TA98
TA1978/pKM101	TA1978/pKM101	TA1978/TA100
CP1	*his*G46 *str*A	G46
CP10	*his*G46 *str*A *gal*1	CP1
JH10	*his*$^+$ *gal*$^+$	G46
JH10—2	JH10/pSL2	CP14 containing pKM101 mutant/JH10
JH10—3	JH10/pSL3	CP14 containing pKM101 mutant/JH10
JH10—4	JH10/pSL4	CP14 containing pKM101 mutant/JH10
JH10—5	JH10/pSL5	CP14 containing pKM101 mutant/JH10
CP14	*his*G46/pKM101	
CP15	CP10/pKM101	
SL352	TA1535/pSL2	TA1535/JH10—2
SL353	TA1535/pSL3	TA1535/JH10—3
SL354	TA1535/pSL4	TA1535/JH10—4
SL355	TA1535/pSL5	TA1535/JH10—5
SL382	TA1538/pSL2	TA1538/JH10—2
SL383	TA1538/pSL3	TA1538/JH10—3
SL384	TA1538/pSL4	TA1538/JH10—4
SL385	TA1538/pSL5	TA1538/JH10—5
SL752	TA1975/pSL2	TA1975/JH10—2
SL753	TA1975/pSL3	TA1975/JH10—3
SL754	TA1975/pSL4	TA1975/JH10—4
SL755	TA1975/pSL5	TA1975/JH10—5
SL782	TA1978/pSL2	TA1978/JH10—2
SL783	TA1978/pSL3	TA1978/JH10—3
SL784	TA1978/pSL4	TA1978/JH10—4
SL785	TA1978/pSL5	TA1978/JH10—5

All strains are *Salmonella typhimurium*

(Lackey *et al.*, 1977; Mortelmans, 1975; Mortelmans *et al.*, 1976).

The ability of pKM101 to enhance mutagenesis and DNA repair was shown to be dependent upon the *recA*$^+$genotype in *Salmonella typhimurium* and upon the *recA*$^+$ *lexA*$^+$ genotype in *Escherichia coli* K-12, suggesting that the plasmid interact with the inducible error-prone DNA repair system of the host cells (Mortelmans *et al.*, 1976).

To determine the gene function of enhanced mutagenesis and repair by the pKM101, mutants of pKM101 modified in these processes were sought. In this paper, induction and characterization of pKM101 mutants is described and is compared with pKM101.

MATERIALS AND METHODS

A. BACTERIAL STRAIN

All strains are derivatives of *S. typhimurium* LT-2 and listed in Table 1. Strain SL352 was obtained by transfer of pKM101 mutant pSL2 to TA1535. Transfer of pKM101 and pKM101 mutant plasmid derived from it followed the procedure of Mortemans *et al.* (1976).

B. INDUCTION OF pKM101 MUTANTS

To isolate plasmid mutants, a strain containing pKM101 was mutagenized with N-methyl-N′-nitro-N-nitrosoguanidine and grown up, and then the mutagenized plasmids were conjugally transferred to a *his*$^-$ *gal*$^-$ strain. These were then screened for modification of ability to reverse *gal*$^-$ mutation by methyl methanesulfonate (MMS) by replica plating on tetrazolium-galactose plates containing methyl methanesulfo nate (Mortelmans. 1975; Park *et al.*, 1975).

The mutant plasmids isolated were then conjugally transferred to *his*$^+$ *gal*$^+$ strain JH10 and subsequently to *his*$^-$ *gal*$^-$ strain TA1975, TA1978, TA1535 and TA1538 on tetrazolium-galactose medium (Lederberg, 1948). Overnight 37°C unshaken broth cultures of donor and recipient were added, 0.1m*l* donor and 1m*l* recipient, into 10m*l* nutrient broth. The mixed culture was incubated at 37°C, for 24 to 30 hours and 0.1m*l* of the unshaken mixed culture were plated on tetrazolium-galactose medium supplemented with 200 μg of ampicillin (Mortelmans, 1975; Mortelmans *et al.*, 1975). *gal*$^+$ strains appeared as white colonies whereas *gal*$^-$ strains formed red colonies. When plasmid were transferred from JH10 to TA1975, TA 1978, TA1535, and TA1538 the frequencies of transfer were determined. The frequency of transfer was expressed as the number of resistant recipient cells per resistant donor cells (Silver *et al.*, 1972).

C. AMPICILLIN RESISTANCE DETERMINATION

Clone to be tended for the ampicillin resistance conferred by pKM101 and pKM101 mutants were spot ted each nutrient agar plate supplemented with a different concentration of ampicillin. A control strain without plasmid was always included. The plates were incubated overnight at 37°C and scored for growth or no growth.

D. SPONTANEOUS ANDCHEMICALLY INDUCED REVERSION OF HISTIDINE AUXOTROPHIC STRAINS TO PROTOTROPHY

To 2m*l* of molten top agar solution (0.6%) at 45°C, 0.1m*l* of overnight nutrient broth culture of the bacterial tester strain and 0.1m*l* of the sample solution to be tested were added. The contents were mixed by vortex mixer and poured on Vogel-Bonner minimal agar plates after incubation for 48 hours at 37°C, colonies were counted.

E. MEASUREMENT OF RATE OF SPONTANEOUS AND UV INDUCED REVERSION OF AUXOTROPHIC MUTANTS TO PROTOTROPHY

An overnight nutrient broth culture, incubated at 37°C, unshaken, of the strain to be tested was washed twice in "C" salts solution (Mortelmans *et al.*, 1976) by centrifuging, discarding the supernatant and resuspending in a volume of "C" salts solution that would give an estimated 2×10^8 cells/m*l*.

Samples (0.1m*l* each) were plated on CY medium supplemented "C" salt solution with nutrient broth, 1% (v/v). The plates were exposed to UV-irradiation and the plates were wrapped in aluminum foil to prevent any pos-

sible photoreactivation. After incubation for two days at 37°C, the plates were scored for the number of colonies, *i.e.* prototrophic revertants (Mortelmans *et al.*, 1976).

F. QUANTITATIVE UV-SURVIVAL DETERMINATION

Serial decimal dilution of overnight standing broth culture were made in nutrient broth.

Drops (0.1m*l*) from appropriate dilution were delivered onto the surface of nutrient agar plates. A sterile glass spreader ensured an even distribution of the bacteria over the surface of the plate.

The plates were exposed to UV-irradiation. The irradiated plates were wrapped in aluminium foil to prevent any possible photoreactiv ation. Colonies were counted in drop areas after overnight incubation at 37°C.

RESULTS

A. THE FREQUENCY OF TRANSFER

The frequency of transfer was determined when plasmids were transmitted from *his*$^+$ *gal*$^+$ strain, JH10, to *his*$^-$ *gal*$^-$ strain, TA1975, TA 1978, TA1535, and TA1538 (Table 2).

Table 2. Transfer frequencies of plasmid pKM101 and pKM101 mutants in *S. typhimurium* TA1975 TA1978, TA1535, and TA1538.

Strain / Plasmid	TA1975	TA1978	TA1535	TA1538
pKM101	3.16×10^{-2}	1.86×10^{-2}	1.05×10^{-2}	1.03×10^{-2}
pSL2	1.73×10^{-2}	1.51×10^{-2}	9.96×10^{-3}	1.08×10^{-2}
pSL3	2.42×10^{-3}	5.35×10^{-3}	1.90×10^{-3}	2.92×10^{-3}
pSL4	9.46×10^{-3}	5.85×10^{-3}	9.65×10^{-3}	5.78×10^{-3}
pSL5	8.76×10^{-1}	3.77×10^{-1}	1.09×10^{-1}	4.32×10^{-2}

As seen in Table 2, the mutant pSL3 was transferred at a slightly lower frequency than pKM101 and other mutants. In contrast, the mutant pSL5 was transferred at a higher frequency. The frequencies of transfer were dependent upon conditions. All plasmids transferred were stable in strains used.

B. AMPICILLIN RESISTANCE DETERMINATION

Ampicillin resistancy of the plasmid mutants was either higher than or the same level as pKM101 (Figure 1). Ampicillin resistancy of the mutant pSL3 is same level as pKM101, whereas all other mutant plasmid had increased ampicillin resistance.

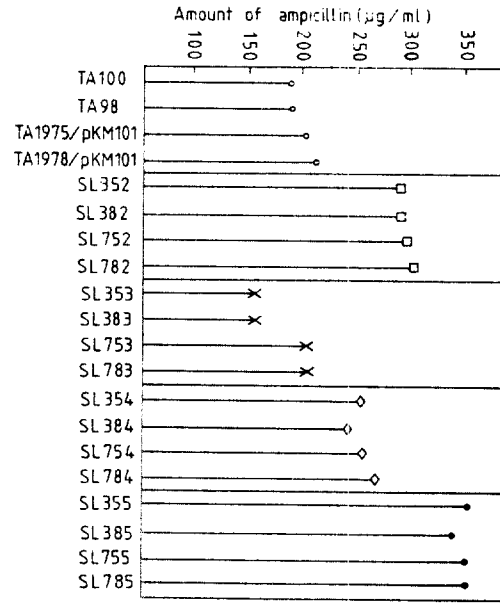

Fig. 1. Ampicillin resistancy of pKM101 mutants

C. EFFECTS ON SPONTANEOUS AND CHEMICALLY-INDUCED REVERSION RATES

The mutant plasmid were tested for their ability to enhance or decrease mutagenesis in the Ames tester system (Ames *et al.*, 1973). For strains containing pKM101 and pKM101

mutants, linear dose response relationships were obtained for reversion of histidine mutation as a function of the amount of mutagen on the plate. As shown in Figure 2 and 4, the pKM101 mutant pSL3 lost its ability to enhance reversion of TA1975 with MMS and 4-NQO, whereas the mutant pSL2 showed only a partial reduction in its ability.

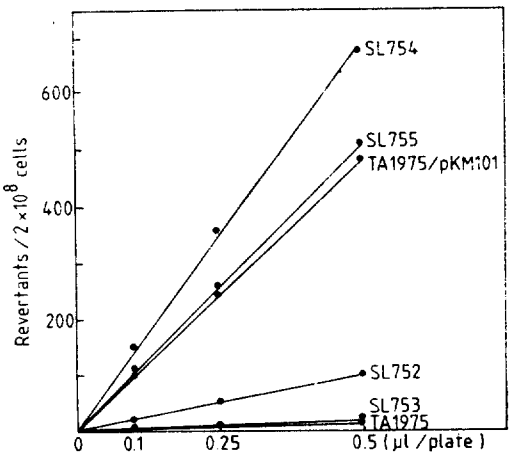

Fig. 2. Effect of pKM101 and pKM101 mutants on MMS mutagenesis

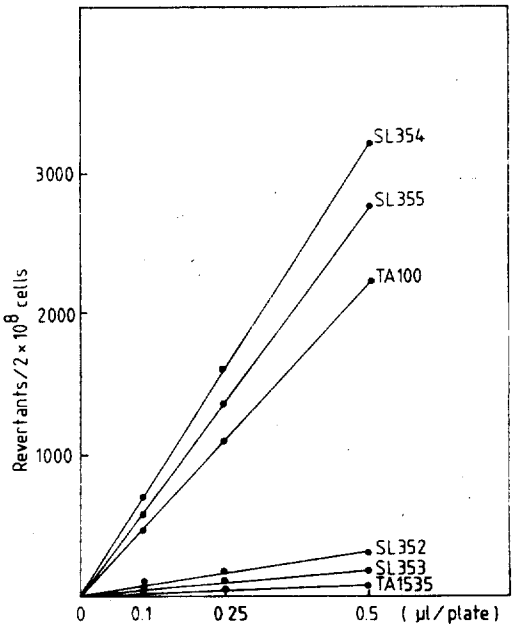

Fig. 3. Effect of pKM101 and pKM101 mutants on MMS mutagenesis

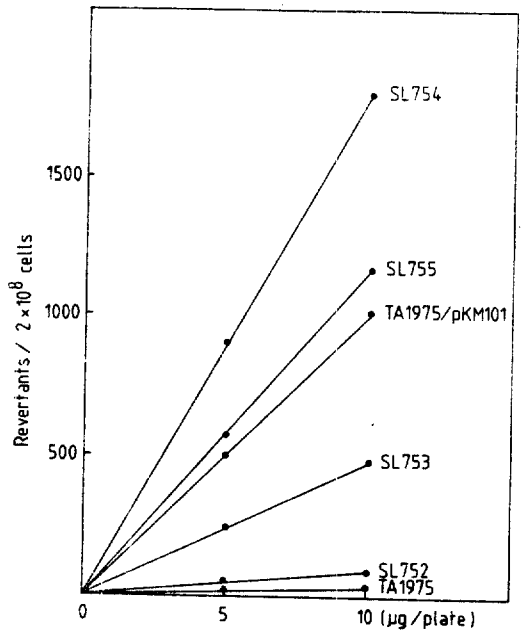

Fig. 4. Effect of pKM101 and pKM101 mutants on 4-NQO mutagenesis

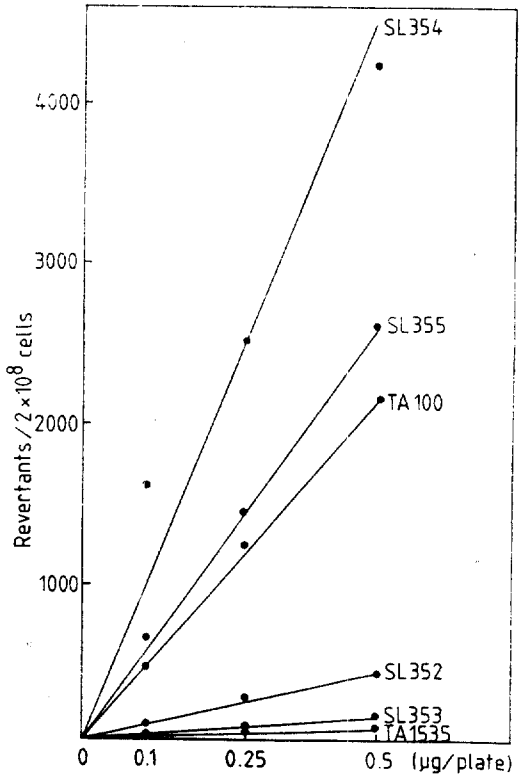

Fig. 5. Effect of pKM101 and pKM101 matants on 4-NQO mutagenesis

The TA1975 and TA1535 locus carries a missense mutation. In contrast, the mutant pSL4 and pSL5 showed an enhancement mutagenesis. Similar and led paralleled results were observed with TA1535 locus by MMS and 4-NQO (Figure 3,5) The TA1535 carries a *vurB*$^-$ mutation. Thus the ability of the pKM101 mutants to enhance or decrease mutagenesis appeared to be independent of the function of the accurate *uvr* repair system.

The pKM101 mutants pSL2, pSL3, pSL4, and pSL5 were also introduced into the frameshift tester strain TA1978 and TA1538 of Ames tester system. The pKM101 causes an enhancement of the reversion to *his*$^+$ in these strains with 4-NQO. The mutant pSL2 and pSL3 caused lower mutagenesis rather than pKM101, whereas the mutant pSL4 and pSL5 enhanced mutagenesis rather than pKM101.

However, a considerable difference was not observed among the pKM101 mutant because 4-NQO is base substitution mutagen (Figure 6,7).

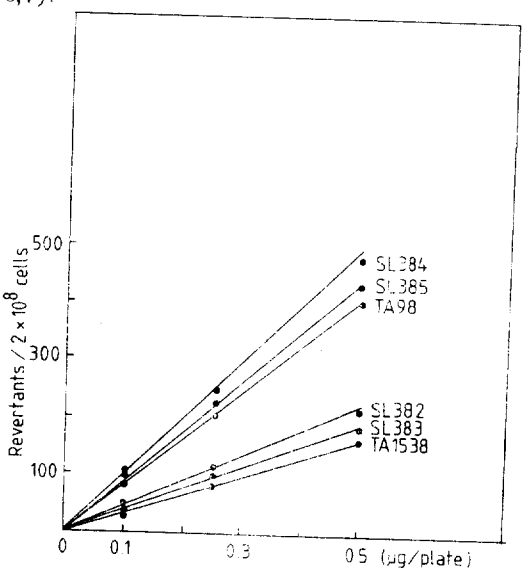

Fig. 6. Effect of pKM101 and pKM101 mutants on 4-NQO mutagenesis

In general, the ability of the plasmids to enhance or diminish the reversion of mutations by chemical was accompanied by the ability to

D. EFFECTS ON THE UV-INDUCED MUTAGENESIS

The mutant plasmid were tested in a histidine-requiring mutant TA1975, which carries missense mutation (Ames *et al.*, 1973; Ames *et al.*, 1975). pKM101 and pKM101 mutants

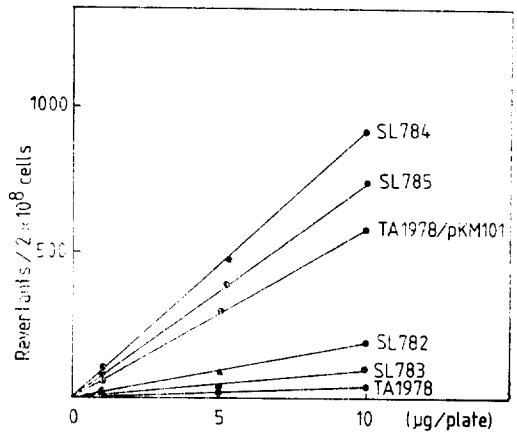

Fig. 7. Effect of pKM101 and pKM101 mutants on 4-NQO mutagenesis

enhance or diminsh the spontaneous reversion (Table 3).

Table 3. Effect of pKM101 and pKM101 mutants on the spontaneous reversion

Strain	His$^+$ revertant/ 10^8 cells	Strain	His$^+$ revertant/ 10^8 cells
AT1975	2	TA1535	14
TA1975/pKM101	18	TA100	145
SL752	15	SL352	63
SL753	5	SL353	16
SL754	31	SL354	225
SL755	22	SL355	180

were introduced to TA1975 by conjugation by mixed growth with JH10/plasmid as described in Materials and Methods. UV-irradiation caused a significant increase in the number of prototrophic revertants in TA1975 strain. The presence of plasmid except pSL3 enhanced this increase (Figure 8).

However, there was no conspicuous difference between pKM101 and pKM101 mutants, pSL2 and pSL5. The mutant pSL4 enhanced

UV-mutagenesis more than the original pKM-101, whereas but the mutant pSL3 lost the mutator effect completely.

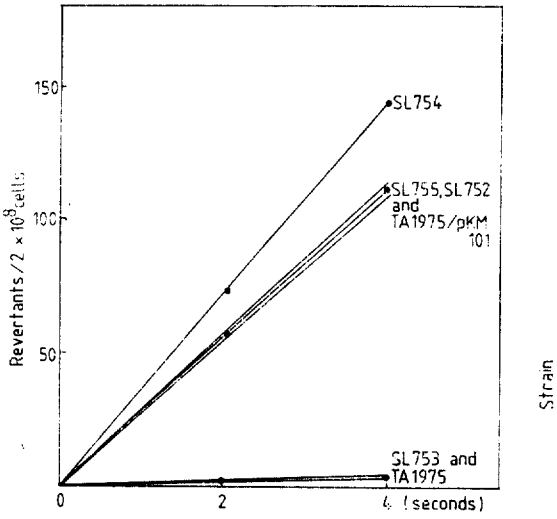

Fig. 8. Effect of pKM101 and pKM101 mutants on UV mutagenesis

E. EFFECTS ON THE CHEMICAL MUTAGEN-INDUCED TOXICITY

Lethal effect of MMS and 4-NQO on R^+ strains(carrying a R plasmid) and R^- strains (without a plasmid) showed only a little differences (Figure 9,10). The mutant pSL4 showed only a little reduction of killing effect by the chemicals compared to that of pKM101.

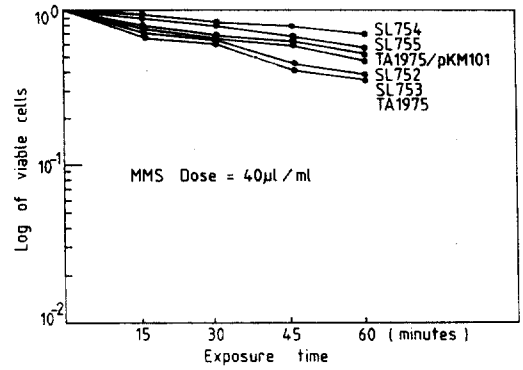

Fig. 9. MMS-dose/log-survival curve

F. EFFECTS ON THE UV-PROTECTION

UV-survival curves of TA1975 containing representative mutant plasmids are shown in Figure 11. The pKM101 mutant pSL3 that have lost most of their ability to enhance mutagenesis have also lost most of their ability to

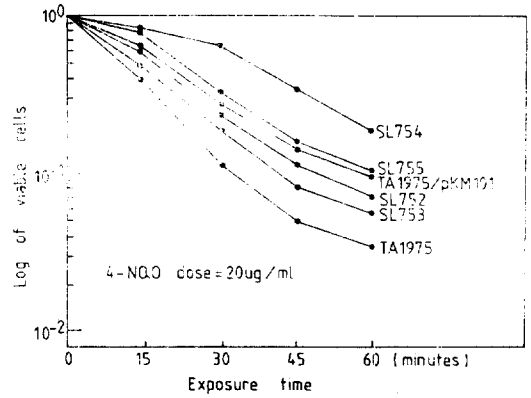

Fig. 10. 4-NQO-dose/log-survival curve

protect the cells against killing by UV. The mutant pSL2, which showed an intermediate level of enhancement of mutagenesis, also imparts an intermediate level of UV-protection to the cells. The pKM101 mutants, pSL4 and pSL5, gave a slightly higher level UV-protection than of pKM101.

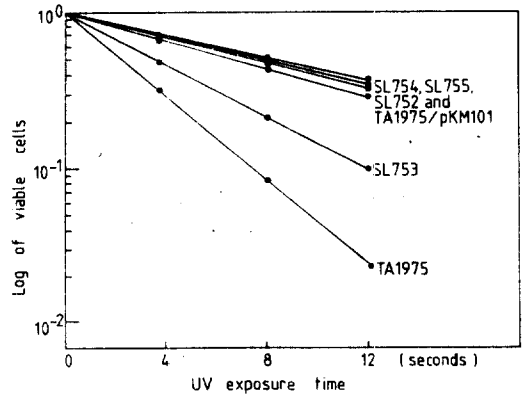

Fig. 11. UV-dose/survival curve

DISCUSSION

Mutants of the plasmid pKM101 which were modified in their ability to enhance mutagenesis have induced and characterized. By use of chemical mutagens and UV, pKM101 mutants

were campared with pKM101 in their ability to enhance mutagenesis and cellular survival. Two mutants of pKM101, pSL2, pSL3 reduced or lost in their ability to enhance reversion by MMS or 4-NQO, whereas two pKM101 mutants, pSL4, pSL5 showed enhancement of reversion by MMS and 4-NQO. In all these pKM-101 mutants the modification in the enhancement of mutation appeared to be qualitatively similar for both MMS and 4-NQO.

The same trend was also observed in both *uvr*B$^+$ and *uvr*B$^-$ strains. Thus the modification of pKM101 mutants to enhance mutagenesis appeared to be independent of the function of the accurate *uvr* repair system. In case of UV-mutagenesis, the results of pKM101 mutation were similar to that of chemical mutagensis. The effects of UV and chemical protection by pKM101 and its mutants showed similar trend to those of mutagenesis. These evidence suggest that there is a close interrelationship between the mutagenesis enhancing and protecting effect of pKM101.

In addition to the abilities to UV and chemical mutagen protect the cell and to enhance mutagnesis, pKM101 is capable of enhancing the spontaneous reversion rate many point mutation and a few frameshift mutations. This effect of pKM101 mutants was also modified in the same fashion. This would imply that the enhancement of spontaneous reversion by pKM-101 is also related to the mechanism by which pKM101 enhances chemical mutagenesis rather that being an independent mutator property incidentally carried on the plasmid. Thus, there appears to be one plasmid-coded gene affecting mutagenesis and repair. However, other known pKM101 function, expression of ampicillin resistance and transmissibility, were affected by other plasmid-coded genes.

摘 要

Mutator effect를 가진 plasmid pKM101을 MNNG처리와 tetrazolium-glactose 배지에서 MMS에 의한 *Salmonella typhimuriun*의 Gal$^-$에서 Gal$^+$로 전환시키는 능력에 따라 pKM101 변이체들을 선택하고 이들의 성질을 chemical과 UV를 사용하여 pKM101과 비교 하였다.

pKM101변이체들은 *Salmonella typhimuriun*내에서 모두 안정하였고 정상적으로 다른 균주로 전달되었으며 ampicillin에 대한 저항성은 원래의 plasmid보다 같거나 높아졌다.

이들중 두 변이체는 chemical mutagen인 MMS와 4-NQO, 그리고 UV에 의한 *Salmonella typhimurium*의 돌연변이율을 모체 pKM101보다 증가시켰으며 다른 변이체들은 이에 대한 효과가 감소 되었거나 전혀 없은 것이었다.

Salmonella typhimurium uvr$^-$ 변이주에 있어서 이들 변이체들의 chemical과 UV에 의한 돌연변이율에 미치는 효과와 치사효과는 모체 pKM101과 비슷한 양상을 나타내었다.

이러한 결과는 plasmid pKM01의 chemical 및 UV mutator effect가 한 유전인자에 의해 결정된다는 것을 시사한다.

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