JOURNAL OF BACTERIOLOGY, Feb. 1968, p. 540-545

Vol. 95, No. 2
Printed in U.S.A.

Properties of Mitomycin C-sensitive Mutants of *Escherichia coli* K-12

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Received for publication 1 December 1967

Strains hypersensitive to mitomycin C (MC) were isolated from *Escherichia coli* K-12 after treatment with nitrosoguanidine. Of 43 MC-sensitive strains tested for their ultraviolet light (UV) sensitivity and for their ability to reactivate UV-inactivated λ phage, 38 were found to be insensitive to UV irradiation and to be able to reactivate UV-irradiated bacteriophage λ. Some properties of the MC-sensitive, *uvr*$^+$ mutants were analyzed. Synthesis of deoxyribonucleic acid (DNA) in MC-sensitive, *uvr*$^+$ mutants was inhibited at a lower concentration of MC than in the wild-type strain. Mutant cells, labeled with ^{3}H-thymidine and then exposed to MC, released radioactivity as low molecular weight compounds. The amount of radioactivity released was the same as that from the wild-type strain. MC-sensitive, *uvr*$^+$ mutants, as well as the corresponding wild-type strain, were equally susceptible to induction of prophage φ80 by UV irradiation. However, MC induction of prophage was achieved in MC-sensitive, *uvr*$^+$ mutants at a lower concentration of the antibiotic than in the wild-type strain. Genetic experiments indicated that a gene controlling MC sensitivity is located close to that determining lactose fermentation of *E. coli*. It is situated on episome F'13, and the wild type is dominant to the MC-sensitive allele.

Mitomycin C (MC), one of a group of alkylating antibiotics, has been reported (5, 6, 14) to cause cross-linking of the complementary strands of deoxyribonucleic acid (DNA). Its effects on bacteria and phages have been found (8–10, 13) very similar to those of ultraviolet light (UV). It has been reported that UV-resistant mutants of *Escherichia coli* are cross-resistant to MC (4, 11) and that the UV-sensitive mutants are more sensitive to the lethal effects of MC (2). Recently, it has been demonstrated that the wild-type strain of *E. coli* contains an efficient mechanism for repair of DNA damaged by exposure of cells to UV or MC (1, 2, 13), whereas UV-sensitive strains lack this mechanism (1, 2, 7, 12). Therefore, it has been assumed that damage produced by UV or by MC might be repaired by the same molecular mechanism (2). Although the UV-sensitive mutants of *E. coli* were shown to be cross-sensitive to MC, there has been no report of MC-sensitive, UV-insensitive mutants of *E. coli*. To study the relationship between sensitivity to UV and MC, strains of *E. coli* hypersensitive to the action of MC were isolated. In this paper, some of the properties of strains sensitive to MC but insensitive to UV will be described, in connection with the mechanism of action of UV irradiation on bacteria.

MATERIALS AND METHODS

Abbreviations. Abbreviations and symbols used for designation of genotype are: *thr*, threonine; *leu*, leucine; *arg*, arginine; *his*, histidine; *met*, methionine; *pro*, proline; *thi*, thiamine; *lac*, lactose; *gal*, galactose; *ara*, arabinose; *xyl*, xylose; *mtl*, mannitol; +, capable of producing or utilizing; −, incapable of producing or using; *str*^{R}, resistant to streptomycin; *mtc*^{S}, sensitive to mitomycin C; *ton*^{S}, *lam*^{S}, sensitive to phages T1 or λ; *tsx*^{R}, resistant to phage T6; *hcr*, host-cell in reactivation.

Bacterial strains. The following strains of *E. coli* K-12 were employed: AB1157 (*thr*$^-$, *leu*$^-$, *pro*$^-$, *his*$^-$, *arg*$^-$, *thi*$^-$, *lac*$^-$, *gal*$^-$, *ara*$^-$, *xyl*$^-$, *mtl*$^-$, *ton*^{S}, *tsx*^{R}, *lam*^{S}, *str*^{R}, *F*$^-$), AB1885 (AB1157 UV-sensitive), and W3747 (*met*$^-$/F'13). Strains AB1157 and AB1885 were kindly supplied by P. Howard-Flanders, Yale University, New Haven, Conn. M5, M18, M22, M28, M29, and M32 were MC-sensitive strains which were isolated from AB1157 by nitrosoguanidine treatment in this laboratory.

Media. Nutrient broth contained (per liter of distilled water) 10 g of polypeptone and 10 g of beef extract (Kyokuto); it was adjusted to *p*H 7.2 with NaOH. For nutrient agar, nutrient broth was solidified with 1.5% agar.

Peptone-agar contained (per liter of distilled water) 10 g of polypeptone, 2.5 g of NaCl and 12 g of agar.

M medium contained (per liter of distilled water) 10.5 g of K_2HPO_4, 4.5 g of KH_2PO_4, 0.05 g of $MgSO_4$, 1.0 g of $(NH_4)_2SO_4$, 0.47 g of sodium citrate, and 2.0

g of glucose. Glucose was sterilized separately and mixed with the medium after cooling. Enriched M medium (EM) consisted of M medium with 2.5 g of Casamino Acids and 0.1 mg of thiamine added per liter.

EM buffer consisted of EM medium in which glucose was eliminated.

EMB lactose-agar contained 0.8% polypeptone, 0.5% yeast extract, 0.04% eosin Y, 0.0065% methylene blue, 0.2% K_2HPO_4, 1% lactose, and 1.5% agar.

Isolation of mutants. Strain AB1157 was grown overnight in nutrient broth, diluted 20-fold to fresh broth, and aerated at 37 C to bring the cells into early log phase. After the cells were washed twice with sterilized saline, they were suspended in 0.2 M acetate buffer (*p*H 5.0), and an aqueous solution of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in the same buffer was added to a final concentration of 250 μg/ml. The cells were incubated for 2 hr at 37 C, centrifuged, and suspended in nutrient broth. After dilution 1:20 in 20 tubes of the same broth, the cells were aerated further for 4 hr. After appropriate dilution, 0.1 ml of the dilution was spread on nutrient agar and the colonies were replicated onto nutrient agar containing 0.1 μg of MC per ml. To eliminate the possibility of picking up the same clone, only one MC-sensitive strain was isolated from a single plate.

Determination of cellular DNA. Portions (10 ml) of the bacterial suspension (3×10^8 cells per ml) were chilled and acidified by the addition of 10 N perchloric acid to give a final concentration of 0.5 N. After standing for 30 min at 0 C, the insoluble residue was collected by centrifugation and heated with 0.5 N perchloric acid at 90 C for 15 min. DNA in the hot perchloric acid-soluble fraction was assayed by the method of Burton (3).

Measurement of radioactivity released from cells labeled with ^{3}H-thymidine after exposure to MC. Overnight cultures were diluted 1:10 into EM medium containing 5 μc of ^{3}H-thymidine (specific activity, 5.0 c/mmole) and 250 μg of deoxyadenosine per ml. They were aerated at 37 C for about 3 hr to bring the cells into early log phase. After the cells were washed twice with EM medium containing 50 μg of nonradioactive thymidine per ml, they were suspended in EM plus thymidine medium and incubated at 37 C for 60 min. After centrifugation, 1 ml of the cells, at a concentration of about 3×10^8 per ml, was exposed to MC for 30 min at 37 C in EM buffer. At the end of incubation a sample of cells was taken for measurement of viable cells; the remaining cells were centrifuged, suspended in EM medium, and aerated for 2 hr at 37 C. Total radioactivity was determined from counting 5-μliter samples of the labeled culture. The cells were acidified by addition of 0.1 ml of 50% trichloroacetic acid and were kept cold for 20 min. After centrifugation, 20 μliters of the supernatant fraction was counted for ^{3}H in a Packard liquid scintillation counter. In one experiment, at 2 hr postincubation, the cells were centrifuged at $2,600 \times g$ for 20 min, and the resultant supernatant fraction was assayed for radioactivity.

Recombination. Cells to be used for mating were grown to the exponential phase (2×10^8/ml) from an inoculum derived from an overnight broth culture. The strains were then mixed in a 200-ml Erlenmeyer flask, with 0.2 ml of the male strain and 2 ml of the female strain used. Mating mixtures were incubated at 37 C without agitation.

Chemicals. MC was supplied by Kyowa Hakko Co. Ltd., Tokyo, Japan, and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine was purchased from Aldrich Chemical Co., Milwaukee, Wis.

RESULTS

UV sensitivity of the MC-sensitive strains. To test the effect of UV irradiation on MC-sensitive strains, two sets of experiments were carried out. In the first set of experiments, strains were grown to logarithmic phase in nutrient broth and a loopful of the culture was streaked onto a peptone-agar plate. The plates were irradiated with an appropriate dose of UV and incubated at 37 C for 24 hr. Of 43 MC-sensitive strains isolated by this method, 5 were shown to be sensitive to UV irradiation and the rest were not. The second series of experiments was based on the fact that UV-irradiated T1 or λ phages form fewer plaques on UV-sensitive mutants than on the corresponding wild type. When λv phage was irradiated with UV to a survival of 10^{-3} and plated on MC-sensitive strains, it was found that the strains which were sensitive to MC but not to UV were able to reactivate UV-inactivated λv phages. The survival of the MC-sensitive strains after treatment with MC and UV, and the survival of UV-irradiated λv phage, when plated on an MC-sensitive strain, are shown in Fig. 1. From these two criteria, i.e., killing by UV and ability to repair UV damage to phage DNA, it became apparent that a majority of the MC-sensitive mutants isolated by nitrosoguanidine treatment were not sensitive to UV irradiation. In the following experiment, MC-sensitive, *uvr*$^+$ mutants were further analyzed to clarify the effect of MC and UV on MC-sensitive strains.

Effect of MC on synthesis and degradation of DNA in MC-sensitive strains. It has been shown that MC exhibits a selective inhibitory action on DNA synthesis without exerting any effect on the synthesis of either RNA or protein in *E. coli* (13). The effect of MC on DNA synthesis in an MC-sensitive strain is shown in Table 1. The synthesis of DNA in an MC-sensitive strain was completely inhibited at a concentration of 0.1 μg/ml, whereas DNA synthesis in the wild-type strain was inhibited only in the presence of 10 μg of MC per ml. The concentration of MC required for the complete inhibition of DNA formation in the MC-sensitive strain was one-twentieth or one-fiftieth of that required for the wild-type strain. This ratio

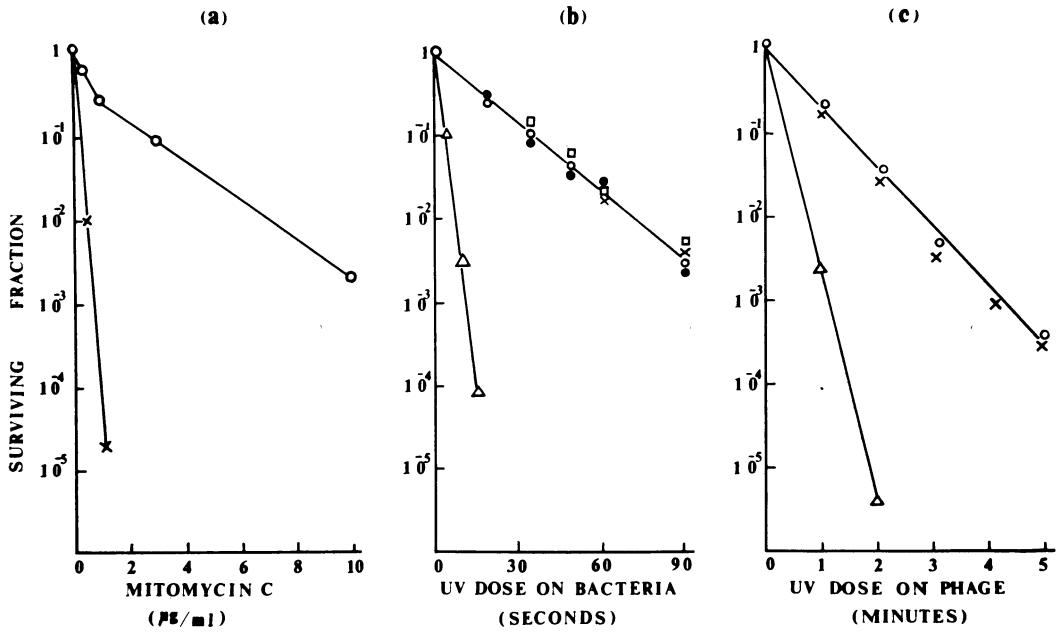

FIG. 1a. *Survival of cells after exposure to various concentrations of mitomycin C. Cells grown in EM medium were harvested in logarithmic phase, washed twice, and suspended in EM buffer. They were exposed to various concentrations of mitomycin C at 37 C for 30 min. After appropriate dilutions, cells were plated on peptone-agar, incubated for about 20 hr at 37 C, and scored for the number of visible colonies formed. Symbols:* ○, *AB1157;* ×, *M32.*

FIG. 1b. *UV survival curves for the mitomycin-sensitive mutants. Cells grown in EM medium were harvested in logarithmic phase, and resuspended in EM buffer. They were exposed to various doses of UV at a distance of 45 cm from a 15-w Toshiba germicidal lamp, diluted, and plated on peptone-agar medium. They were incubated for about 20 hr at 37 C and scored for the number of viable colonies formed. Symbols:* ○, *AB1157;* △, *AB1885;* ●, *M22;* □, *M28;* ×, *M32.*

FIG. 1c. *UV survival curve for λv phage plated on mitomycin C-sensitive mutants. Phage were irradiated in EM buffer at pH 7.2 and plated on peptone-agar plates with soft agar seeded with the indicated strains of bacteria. Symbols:* ○, *AB1157;* △, *AB1885;* ×, *M32.*

correlates well with the relative amount of MC required to produce lethal effects on these strains. Furthermore, as shown in Table 1, the amount of DNA in the MC-sensitive strain was decreased when the cells were incubated with 1 μg of MC per ml for 30 min, indicating that MC induced the degradation of DNA in the MC-sensitive strain.

If this degradation were due to the mechanism for excision of UV photoproducts, the repair of MC damage to DNA in MC-sensitive strains should be normal. To confirm this, an experiment was performed to test the effect of MC on the release of radioactive materials from DNA which was prelabeled with ^{3}H-thymidine. As shown in Table 2, upon incubation with MC, about 30% of the radioactive materials were released from the MC-sensitive strain into the soluble fraction. No release of radioactivity was observed in the UV-sensitive strain. These results suggest that MC-sensitive strains contain the mechanism which is thought to be involved in the removal from DNA of the products formed by exposure of cells to MC or to UV.

Induction of prophage by MC and UV in a φ80 lysogenic MC-sensitive strain. The experiments presented above indicate that MC acts more efficiently on both the survival and DNA synthesis of MC-sensitive strains than of the wild-type strain. Therefore, one would expect that the induction of phage in lysogenic MC-sensitive strains would be achieved at a lower concentration of MC than that required in the lysogenic wild-type strain. To test this possibility, an MC-sensitive strain, a UV-sensitive strain and a wild-type strain were lysogenized with temperate phage φ80 and assayed for the induction of phage by MC or UV. Cells were grown to a logarithmic phase of growth, exposed to various concentrations of MC or doses of UV, and then plated on an indicator strain to determine the number of

TABLE 1. *Relative amount of DNA in strains of Escherichia coli treated with mitomycin C*[a]

Strain	Concn of mitomycin C (μg/ml)					
	0	0.05	0.1	1	5	10
AB1157.....	1.3	—	1.3	1.35	1.1	0.87
AB1885.....	1.3	—	0.96	0.95	1.0	—
M32.....	1.3	1.1	0.93	0.61	—	0.74

[a] Strains of *E. coli* grown to logarithmic phase were harvested by centrifugation and washed with EM buffer. The cells in EM medium were aerated at 37 C for 30 min in the presence of various concentrations of MC. After 0 and 30 min of incubation, 10 ml of the culture was removed for measurement of the amount of DNA. The relative increase or decrease in the amount of DNA (amount of DNA at 30 min/amount of DNA at 0 min) is shown.

induced cells. As shown in Fig. 2, the number of induced cells in an MC-sensitive strain increased rapidly with increasing concentrations of MC and reached a maximum at a concentration of 0.2 μg/ml. In the corresponding wild-type strain, the number of induced cells increased rather gradually until the concentration of MC reached 5 μg/ml. However, with UV induction, no difference in the number of plaque-forming centers was observed between the lysogenic MC-sensitive strain and the corresponding wild-type strain. It is also shown in Fig. 2 that the maximal induction of phage in the lysogenic UV-sensitive strain was attained at a lower concentration of MC than in the wild-type strain.

Location of gene controlling MC sensitivity. To determine where the *mtc* gene was situated on the *E. coli* chromosome, an MC-sensitive strain was crossed with Hfr Cavalli. Mating was interrupted at 30 min and mtc^+ str^R recombinants were selected. After purifying the recombinants on the same plate, the colonies were tested for unselected markers by the replica plate method. As shown in Table 3, 99% of the recombinants were lac^+ and pro^+, whereas none received the donor gene his^+ or gal^+. This suggests the *mtc* gene is located close to the *pro* and *lac* genes.

Its location was confirmed by crosses between Hayes or Cavalli Hfr and MC-sensitive strains, in which lac^+ recombinants were selected. In these crosses, mating was interrupted at 30 min by blending, and recombinants were selected on EMB-lac-SM medium. Lac^+ colonies were picked up on the same plate and replica-plated onto the agar medium containing MC. As shown in Table 4, 92% of the lac^+ recombinants received the *mtc* marker from the donor strain.

In *E. coli*, a fragment of the bacterial chromosome sometimes attaches to sex factor F. F'13 is one such episome on which the bacterial genes *lac*, *pro*, *pho*, and *pur* are found. Since the *mtc* gene was located close to the *lac* gene, it was assumed that the *mtc* gene might also be found on episome F'13. To test this possibility, overnight cultures of strain W3747 and mtc^S strains (M18, M28, M32) were mixed in nutrient broth at a cell

TABLE 2. *Colony-forming ability and DNA degradation of Escherichia coli treated with mitomycin C*

Expt	Strain	Concn of mitomycin C (μg/ml)	Fraction of cells surviving	Percentage of total radioactivity in soluble fraction
1[a]	AB1157	0	1	4.4
		0.5	8.7×10^{-2}	20.0
		1	8.5×10^{-3}	30.8
		5	6.4×10^{-4}	13.5
	AB1885	0	1	6.0
		0.1	5.7×10^{-3}	6.7
		0.5	7.2×10^{-5}	8.3
		1	4.6×10^{-5}	9.4
	M28	0	1	5.7
		0.1	1.5×10^{-1}	10.0
		0.5	3.0×10^{-4}	33.6
		1	3.3×10^{-4}	20.8
	M29	0	1	7.5
		0.1	7.7×10^{-2}	8.3
		0.5	3.2×10^{-4}	24.3
		1	1.2×10^{-4}	27.0
2[a]	M5	0	1	3.9
		0.1	3×10^{-1}	7.1
		0.5	2.8×10^{-3}	41.2
		1	10^{-4}	28.0
	M18	0	1	4.6
		0.1	2.6×10^{-1}	10.1
		0.5	1.7×10^{-4}	32.1
		1	10^{-4}	25.4
	M32	0	1	5.9
		0.1	4×10^{-1}	33.2
		0.5	3.4×10^{-4}	39.2
		1	10^{-4}	24.3
3[b]	AB1157	0	1	6.8
		0.5	8.7×10^{-2}	21.1
		1	1.1×10^{-2}	46.8
		5	1.0×10^{-5}	18.7
	AB1885	0	1	9.1
		0.5	1×10^{-3}	11.2
		1	1×10^{-5}	12.8
		5	10^{-5}	12.9
	M32	0	1	6.8
		0.5	2.4×10^{-4}	46.5
		1	10^{-5}	33.6
		5	10^{-5}	24.4

[a] At 2 hr postincubation, radioactivity in the trichloroacetic acid-soluble fraction was counted.

[b] At 2 hr postincubation, radioactivity released into the medium was counted

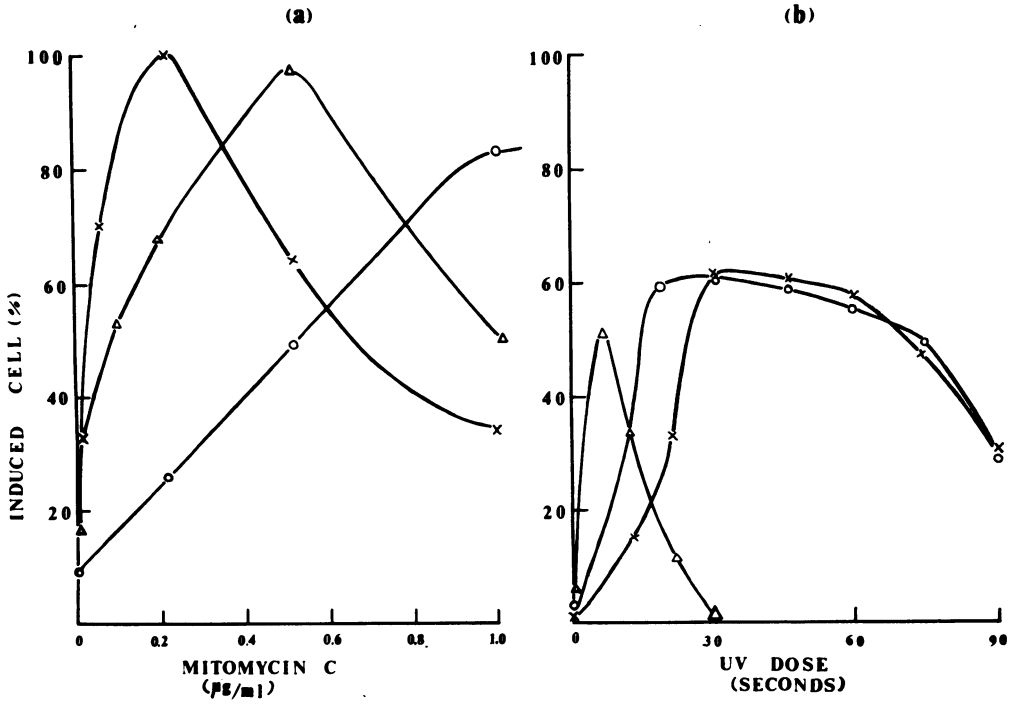

FIG. 2a. *Induction by mitomycin C of prophage φ80 in a mitomycin C-sensitive mutant. Lysogenic cells, grown in EM medium, were harvested in logarithmic phase, washed twice, and resuspended in EM buffer at a concentration o, 2 × 10⁸ cells per ml. The cells were diluted 100-fold in EM buffer and exposed to various concentrations of MC at 37 C. After incubation for 20 min, they were diluted and plated with soft agar with the appropriate indicator strains. Symbols: ○, AB1157; △, AB1885; ×, M32.*

FIG. 2b. *Induction by UV of prophage φ80 in the mitomycin C-sensitive mutant. Lysogenic cells, harvested and washed as described in Fig. 2a, were suspended in 0.9% NaCl and exposed to various doses of UV at a distance of 45 cm from 15-w Toshiba germicidal lamp. After appropriate dilution, the induced cells were measured by plating with soft agar on the appropriate indicator strains. Symbols: ○, AB1157 (φ80); △, AB1885 (φ80); ×, M32 (φ80).*

TABLE 3. *Frequency of occurrence of unselected donor markers in mtc⁺ strᴿ recombinants from three crosses*[a]

Cross	No. of *mtc⁺ strᴿ* colonies tested	No. of unselected donor markers				
		lac⁺	*pro⁺*	*thr⁺ leu⁺*	*his⁺*	*gal⁺*
1	100	100	100	11	0	0
2	100	100	100	9	0	0
3	103	102	102	13	0	0

[a] Crosses were as follows: (1) Hfr Cavalli *strˢ* × M18, (2) Hfr Cavalli *strˢ* × M28, (3) Hfr Cavalli *strˢ* × M32. Mating was interrupted at 30 min.

TABLE 4. *Frequency of occurrence of unselected donor markers in lac⁺ strᴿ recombinants from two crosses*[a]

Cross	No. of colonies tested	No. of unselected donor markers				
		mtc⁺	*pro⁺*	*thr⁺ leu⁺*	*his⁺*	*gal⁺*
1	179	172	177	99	0	0
2	182	170	182	151	0	0

[a] Crosses were as follows: (1) Hfr Cavalli × M32 (2) Hfr Hayes × M32.

density of about 10^5 per ml and incubated at 37 C for 8 hr. After appropriate dilution, the cells were plated on EMB-Lac-SM plates and incubated for about 20 hr. Lactose-positive colonies were picked, purified, and tested for their sensitivity to MC. It was found that all of the lactose-positive colonies thus obtained were no longer MC-sensitive. They grow in the medium containing MC as well as the wild-type strain did. It was also found that 100 *lac⁻* colonies, which arose spontaneously or after acridine orange treatment from an F ductant, M32/F'13, became sensitive to the action of MC. These results indicate that the gene controlling MC sensitivity is located on episome F'13 and the wild-type allele is dominant to the MC-sensitive gene.

DISCUSSION

It is known that pyrimidine dimers are formed in DNA with a high yield when bacteria are exposed to UV. Usually, bacteria contain efficient mechanisms for removing lethal UV damage to DNA as acid-soluble oligonucleotides (1, 12). In UV-sensitive, *hcr*$^-$ mutants, this mechanism for DNA repair is deficient (1, 12). Recently, Boyce and Howard-Flanders showed that the *hcr*$^-$, UV-sensitive mutants are also sensitive to the bactericidal action of MC and are incapable of removing MC damage to DNA as acid-soluble oligonucleotides (2). The MC-sensitive mutants analyzed in this paper, however, were not sensitive to UV and were able to reactivate UV-inactivated λv phages. The mutants were also shown to contain the normal mechanism for excision of MC damaged DNA (Table 2), indicating that the *mtc* gene controls some process other than that for the excision of pyrimidine dimers in DNA. Since the *mtc* gene was shown to locate close to the *lac* gene of *E. coli*, one could conclude that there are at least two types of gene which control the MC sensitivity of *E. coli*, i.e., one which participates in the repair of UV and MC damage in DNA and another which controls the MC, but not the UV, sensitivity of the bacteria.

ACKNOWLEDGMENTS

The author is grateful to J. Kawamata for his encouragement in this work and to S. Goodgal for his help in the preparation of the manuscript. This investigation was supported by grants from the Ministry of Education, Japan, and from the Jane Coffin Childs Memorial fund.

LITERATURE CITED

1. BOYCE, R. P., AND P. HOWARD-FLANDERS. 1964. Release of ultraviolet induced thymine dimers from DNA in *E. coli* K-12. Proc. Natl. Acad. Sci. U.S. **51**:293–300.
2. BOYCE, R. P., AND P. HOWARD-FLANDERS. 1964. Genetic control of DNA breakdown and repair in *E. coli* K-12 treated with mitomycin C or ultraviolet light. Z. Vererbungslehre **95**:345–350.
3. BURTON, K. 1956. A study of the conditions and mechanism of the diphenylamin reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. **62**:315–323.
4. GREENBERG, J., J. D. MANDEL, AND P. L. WOODY. 1961. Resistance and cross-resistance of *Escherichia coli* mutants to antitumour agent mitomycin C. J. Gen. Microbiol. **26**:509–520.
5. IYER, V. N., AND W. SZYBALSKI. 1963. A molecular mechanism of mitomycin action: Linkage of complementary DNA strands. Proc. Natl. Acad. Sci. U.S. **50**:355–362.
6. IYER, V. N., AND W. SZYBALSKI. 1964. Mitomycin and porfiromycin: Chemical mechanism of activation and cross-linking of DNA. Science. **145**:55–56.
7. OKUBO, S., AND W. R. ROMIG. 1966. Impaired transformability of *Bacillus subtilis* mutant sensitive to mitomycin C and ultraviolet radiation. J. Mol. Biol. **15**:440–454.
8. OTSUJI, N., AND S. OKUBO. 1960. Reactivation of ultraviolet and nitrous acid-inactivated phages by host cells. Virology **12**:607–609.
9. OTSUJI, N., M. SEKIGUCHI, T. IIJIMA, AND Y. TAKAGI. 1959. Induction of phage formation in the lysogenic *Escherichia coli* K-12 by mitomycin C. Nature **184**:1079–1080.
10. REICH, E., J. SHATKIN, AND E. L. TATUM. 1961. Bactericidal action of mitomycin C. Biochim. Biophys. Acta **53**:132–149.
11. SEKIGUCHI, M., AND Y. TAKAGI. 1960. Effect of mitomycin C on the synthesis of bacterial and viral deoxyribonucleic acid. Biochim. Biophys. Acta **41**:434–443.
12. SETLOW, R. B., AND W. L. CARRIER. 1964. The disappearance of thymine dimers from DNA: An error-correcting mechanism. Proc. Natl. Acad. Sci. U.S. **51**:226–231.
13. SHIBA, S., A. TERAWAKI, T. TAGUCHI, AND J. KAWAMATA. 1959. Selective inhibition of formation of deoxyribonucleic acid in *Escherichia coli*. Nature **183**:1056–1057.
14. WEISSBACH, A., AND A. LISIO. 1965. Alkylation of nucleic acids by mitomycin C and porfiromycin. Biochemistry **4**:196–200.