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Mitomycin C-sensitive Mutant of *Escherichia coli* K-12

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A mitomycin C (MC)-susceptible mutant was found by accident in an aged stock culture of *Escherichia coli* K-12 strain W3110 (F^-, λ-sensitive). When MC was added to a log-phase culture of this mutant strain (W3110 MCr^-) in tryptone broth, to a final concentration of 1 μg/ml, the growth-inhibitory effect of MC began to appear after a 30-min lag period. The growth rate decreased gradually, and within 90 min it reached a value of about one-tenth of that in a control culture without MC. In a parallel experiment with the wild type (W3110 MCr^+), the growth of the cells was not affected appreciably at this concentration of MC. About 10 μg of MC per ml was necessary to produce a similar degree of growth inhibition.

It is known that the ultraviolet light (UV)-sensitive mutants so far isolated are susceptible to MC (R. P. Boyce and P. Howard-Flanders, Z. Vererbungsl. **95**:345, 1964). However, the sensitivity of this mutant to the killing action of UV was the same as that of the wild type (Table 1). It is assumed, therefore, that the mechanism causing the high susceptibility to MC in this mutant differs from those in the known UV- or MC-susceptible mutants.

TABLE 1. *Survival of cells after exposure to ultraviolet light*

Strain	Survival (%)[a]
W3110 MCr^+	59
W3110 MCr^-	74
AB1157[b]	75
AB1886[c]	1.1×10^{-3}

[a] Log-phase cells in T-broth, which contains 1% tryptone broth, 10^{-3} M $MgCl_2$, and 1 μg of thiamine per ml, were harvested and suspended in 0.01 M tris(hydroxymethyl)aminomethane-chloride buffer (*p*H 7.4) supplemented with 10^{-3} M $MgCl_2$ to give a cell concentration of 2×10^8 cells/ml. A 5-ml amount of suspension in a petri dish was irradiated with a Toshiba germicidal lamp (15 w) for 10 sec at a distance of 45 cm.

[b] A derivative of *Escherichia coli* K-12; UVr^+, λ^s.

[c] A UV-sensitive derivative of AB1157 (P. Howard-Flanders, R. P. Boyce, and L. Theriot, Genetics **53**:1119, 1966).

In further studies, this mutant was also found to be more sensitive than the original strain to the growth-inhibitory action of acridine orange, methylene blue, and triphenyl tetrazolium chloride (Table 2). It was previously reported by H. Nakamura (J. Bacteriol. **90**:8, 1965) and by Y. Sugino (Genet. Res. **7**:1, 1966) that the resistance of *E. coli* to methylene blue and acridine dyes was determined by a genetic locus which was mapped between the *lac* and *pur* loci. Sugino also reported that the gene in question was carried by F'13, a sex-factor which contained a small fragment of bacterial chromosome including the *lac* and *pur* loci (Y. Hirota and P. H. A. Sneath, Japan J. Genet. **36**:307, 1961). Since our mutant was sensitive to methylene blue and

TABLE 2. *Growth response of strains W3110 MCr^+ and W3110 MCr^- to various chemicals*[a]

Chemical	Concn (μg/ml)	Growth[b]	
		W3110 MCr^+	W3110 MCr^-
Mitomycin C	0.01	+	+
	0.05	+	−
	0.1	+	−
	0.5	−	−
	1.0	−	−
Methylene blue	5	+	−
	10	+	−
	20	+	−
	50	+	−
Acridine orange	20	+	+
	50	+	−
Triphenyl tetrazolium chloride	50	+	+
	100	+	−

[a] An overnight culture grown at 37 C in T-broth was diluted 10^4-fold with the same broth, and cells were streaked by means of a platinum loop on the tryptone plates containing the indicated concentration of chemicals.

[b] Symbols: +, approximately 200 colonies appeared in a streak; −, no colonies.

acridine dyes simultaneously, it was suspected that the sensitivities to all these chemicals, including MC, were due to a pleiotropic effect of a single mutation whose mutational site was in the gene reported by Nakamura and Sugino. This assumption was supported by the following experiments. MC-resistant mutants were obtained at a frequency of about 10^{-6} by plating an overnight culture of W3110 MCr$^-$ on tryptone-agar containing 0.1 μg of MC per ml. Such mutants regained resistances to the other three chemicals. Similarly, a reverse mutant for each of these three chemicals was isolated. The respective revertants were also resistant to all the other chemicals, as was the wild type. In a second experiment, the episome from W3747 (met$^-$) carrying F'13 was transferred to W3110 MCr$^-$. The donor strain was eliminated by the amino acid requirement. Among 52 independent colonies tested, 32 colonies which did not carry F'13 were sensitive to all of the four chemicals. The remaining 20 colonies carrying F'13 were resistant to all the chemicals simultaneously. It is assumed, therefore, that the high sensitivity of W3110 MCr$^-$ to MC is due to a point mutation in the same locus as reported by Nakamura and Sugino.

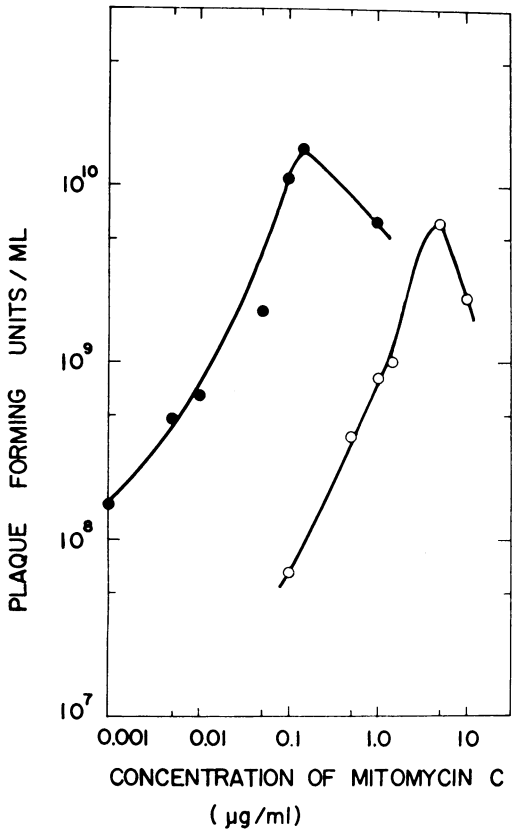

FIG. 1. *Effect of mitomycin C on the induction of prophage λ in strains W3110 MCr$^+$(λ) and W3110 MCr$^-$(λ). Cells were grown at 37 C in tryptone broth containing 10^{-3} M $MgCl_2$ and 1 μg of thiamine per ml. When the cell concentrations of W3110 MCr$^+$(λ) and W3110 MCr$^-$(λ) reached 1.0 × 10^8 cells/ml and 1.4 × 10^8 cells/ml, respectively, cultures were treated with various concentrations of mitomycin C for 2 hr, followed by the addition of chloroform. Plaque titers were determined on λ-sensitive Escherichia coli C600. Symbols: ○, W3110 MCr$^+$(λ); ●, W3110 MCr$^-$(λ).*

The effect of MC on the induction of the prophage was studied by using a λ-lysogenic derivative of this mutant (Fig. 1). The optimal concentration of MC for maximal phage yields with W3110 MCr$^-$(λ) was less than one-tenth of that required with W3110 MCr$^+$(λ). As shown in Fig. 1, the patterns of phage production in the two strains were similar. These observations, together with the results presented above, suggest that the high susceptibility of the mutant to MC may be due to a defect in either "permeability barrier" or "inactivation system" of the cell to the antibiotic. A similar suggestion has been made by Sugino to explain sensitivity to acridine dyes (Genet. Res. 7:1, 1966).

After this work was completed, I learned that a similar mutant was isolated from *E. coli* K-12 strain AB1157 by Nozomu Otsuji, Research Institute of Microbial Diseases, Osaka University (*personal communication*).

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