STRAINS OF *ESCHERICHIA COLI* HYPERSENSITIVE TO REPRESENTATIVE CARCINOSTATIC AND CARCINOGENIC AGENTS

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In recent years studies have been done to devise simple procedures to detect potential carcinogens. The most widely used method among them is the one developed by AMES *et al.*, which is based on back mutation from histidine auxotrophy to prototrophy in strains of *Salmonella typhimurium*[1,2]. Using this system, they tested more than a hundred carcinogens and found strong correlation between carcinogenicity and mutagenicity. Other systems devised for detection of potential carcinogens are the Rec assay by a Rec$^-$ strain of *Bacillus subtilis*[3] and the Inductest which is based on lysogenic induction of prophage λ in *Escherichia coli*[4]. These assay systems may also be applicable for screening of substances which have antitumor activity, since carcinogenic substances often display carcinostatic activity,[5,6] and *vice versa*. For example, antibiotics actinomycin and mitomycin C (MMC), which were originally isolated as antitumor and cytotoxic agents, were also proved to have carcinogenic potency[7,8,9].

Depending on these facts, we tried to construct a strain of *E. coli* with triple mutations in genes, *uvrA*, *ruv* and *tolC* (same as *mtcB*) which might be suitable for detection of substances having carcinostatic activity and/or carcinogenic potency. Each single mutant used in this experiment has the following properties. The ultraviolet light (UV)-sensitive *uvrA* mutant is defective in an initial step of excision of damages on deoxyribonucleic acid (DNA) produced by UV[10] or chemicals acting like UV, and is also sensitive to these substances[11,12,13]. The *ruv* mutant, although normal in ability for excision repair for DNA damage, is sensitive to UV, MMC and nalidixic acid[14]. The *uvrA ruv* strain is more sensitive to UV irradiation than the strain with single mutation in the corresponding gene.[15] The *mtcB* mutant, which was originally isolated as an MMC-sensitive mutant, seems to be identical to the *tolC* mutant, since it is resistant to colicin E1 and sensitive to sodium dodecylsulfate (SDS), sodium deoxycholate, basic dyes and bleomycin[16,17]. The strain with triple mutations in *uvrA*, *ruv* and *tolC* (*mtcB*) was prepared as follows: A strain BE5525 (*uvrA*, *ruv*-9) was plated on L-medium agar (Tryptone, 10 g; yeast extract, 2.5 g; NaCl, 5 g; pH 7.2 and 1.5% agar) with colicin E1 and incubated overnight. Colonies grown on the plate were purified by single colony isolation and each single colony was streaked successively on two L-medium agars, one with and the other without SDS (100 μg/ml). Since it was certain that colicin E1-resistant, SDS-sensitive cells carried *tolC* mutation, colonies on the SDS-free plate which corresponded to SDS-sensitive were picked up. The presence of the *tolC* mutation was confirmed by P1 transduction using the presumptive triple mutant as a donor and strain AT2446 (*metC*) as a recipient. The *metC*$^+$ transductants were found to have the *tolC* mutation at the expected frequencies of about 17%[16]. Genetic characters and source or derivation of the bacterial strains used in this experiment are listed in Table 1.

The compounds, BLM, MMC, carboquone (CBQ) and adriamycin (ARC), which are clinically used in Japan as carcinostatic agents[18,19,20,21], and 4-nitroquinoline-1-oxide (4NQO) and furylfuramide (AF2), both of which are known as potent carcinogen and mutagen[11,12,22,23,24], were used for testing the validity of the present assay system.

As seen in Table 2, an *uvrA* strain, AB1886, was about 2 to 10 times more sensitive than the parent wild type strain, AB1157, to MMC, CBQ, AF2 and 4NQO, but was equally sensitive to BLM. The fact that mutant AB1886 had the same sensitivity as the parent strain to BLM indicates that the lesion in DNA produced by BLM is not repaired by a mechanism responsible for excision of pyrimidine dimers on DNA. The *ruv* mutant, BE5036, was more sensitive to all of the compounds tested than the parent strain and so was the *recA* mutant, AB2463. The *tolC* (*mtcB*) mutant, BEM11, was more sensitive than its parent strain to BLM, MMC, CBQ and ARC,

Table 1. Bacterial strains used.

Strains	Relevant genotype	Other markers[a]	Source or derivation
AB1157	wild	F⁻*thr-1, leu-6, proA2, his-4, thi-1, argE3, lacY1, galK2, ara-14, xyl-5, mtl-1, tsx-33, strA31, sup-37*	P. HOWARD-FLANDERS
AB1886	*uvrA*	As AB1157, also *uvrA*	P. HOWARD-FLANDERS
AB2500	*uvrA*	As AB1886, also *thyA*	P. HOWARD-FLANDERS
BE5036	*ruv*	As AB1157, also *ruv-9*	AB1157 by MNNG[b] 14)
BEM11	*tolC*	As AB1157, also *tolC* (*mtcB*)	AB1157 by MNNG 16)
BE5525	*uvrA ruv*	As AB1886, but *ruv-9, his*⁺	BE5471×AB1886 15)
BE1186	*uvrA ruv tolC*	As BE5525, also *tolC*	This paper
AB2463	*recA*	As AB1157, also *recA13*	P. HOWARD-FLANDERS
BE1166	*uvrA recA*	As AB2500, but *recA1, thy*⁺	KL16-99×AB2500 15)
BE5471	*ruv*	Hfr *ruv-9*	
KL16-99	*recA*	Hfr *recA1*	B. LOW
AT2446	wild	Hfr *metC69, thi*	B. BACHMANN

a) Genetic nomenclature is from B. BACHMANN, *et al.*[17]
b) Abbreviation: MNNG; N-methyl-N′-nitro-N-nitrosoguanidine

Table 2. Minimal inhibitory concentration of test compounds to various strains of *E. coli*.[a]

Strains	Relevant genetic character	Minimal inhibitory concentration (μg/ml)					
		BLM[b]	MMC	CBQ	ARC	AF2	4NQO
AB1157	wild	1.0	5.0	1,000	>200	3.0	30
AB1886	*uvrA*	1.0	3.0	300	>200	0.3	10
BE5036	*ruv*	0.3	3.0	300	200	0.3	10
BEM11	*tolC*	0.1	1.0	500	10	3.0	30
BE1186	*uvrA ruv tolC*	0.01	0.1	3	3	0.1	1
AB2463	*recA*	0.3	1.0	300	200	0.1	10
BE1166	*recA uvrA*	0.3	1.0	300	30	0.01	1

a) Agar plates were prepared in Petri dishes, 6 cm diameter, by pouring 10 ml of L-medium agar containing a series of dilutions of test compound into each plate. A loopful of bacterial cultures grown to log phase in L-medium was streaked on the agar plate and incubated at 37°C for about 22 hours.
b) Abbreviations: BLM, bleomycin; MMC, mitomycin C; CBQ, carboquone; ARC, adriamycin, AF2, furylfuramide; 4NQO, 4-nitroquinoline-1-oxide.

but showed no change in sensitivity to AF2 and 4NQO. Sensitivity of the *tolC* mutant, BEM11, to MMC, CBQ and ARC may be caused by the quinone moiety in their molecular structures, since it is common to these three compounds. It is unclear at the moment which chemical moiety in the BLM molecule is responsible for the increased sensitivity of this mutant strain.

The triple *uvrA ruv tolC* mutant, BE1186, was the most sensitive among the strains tested. It was 100, 50, 300 and 70 times more sensitive than the parent strain to BLM, MMC, CBQ and ARC, respectively. Strain, BE1186, was more sensitive to these compounds than the *uvrA recA* strain, BE1166, which is defective in excision and post-replication repair, and is killed by the presence of one pyrimidine dimer per chromosome.[10]

Since the *uvrA ruv tolC* mutant is very sensitive to substances which produce lesions in DNA, it may be useful for screening substances which are carcinogenic or carcinostatic, especially those structurally related to compounds such as BLM, MMC, CBQ and ARC. This hypersensitive strain is also suitable as a sensitive bioassay system for measuring these compounds in blood or urine. We may also use the strains described

in this paper to deduce the action spectrum of unknown chemical compounds, which are inferred to interact with the DNA molecule, since these strains are defective in different genes concerned with repair of damages in DNA[10,13,14,16,22,23].

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