Method for Selection of Mutagen-sensitive Mutants of *Escherichia coli*

Takao OHISHI and Shigezo UDAKA

Faculty of Agriculture, Nagoya University,
Chikusa, Nagoya 464, Japan

Received August 8, 1977

The usefulness of the bacterial test system for screening environmental mutagens (which are mostly carcinogens) has been well documented.^{1,2)} Bacterial tester strains have mutations of *uvr*,³⁾ *polA*,⁴⁾ *rec*⁵⁾ and combination of some of these mutations.⁶⁾ Although the currently available tester strains are very sensitive to a wide range of mutagens, there still seem to be some mutagenic substances to which none of the known strains are sensitive enough for their assay.

In this paper, we describe a method for efficiently selecting mutants of *E. coli* K-12 which are highly mutable by a low concentration of the particular mutagen. Some of the mutagen-sensitive mutants obtained by such a method may well be valuable as new tester strains.

Attempt was made to isolate mutants preferentially sensitive to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG) mutagenesis. *E. coli* W3876LTL (*F*⁻ *lac* *mal* *str*^r *lys* *leu* *trp*) was used as the parental strain. The *lys* and *leu* auxotrophies could revert to prototrophies by 2-aminopurine or NG, and therefore they appeared to be transition-type point mutations. First the bacteria were mutagenized with 300 µg of NG per ml by a conventional procedure.⁷⁾ After the mutagenized cells were further cultivated aerobically for 3 to 5 hr,

a small amount of NG; i.e., 0.2 µg per ml, which could hardly induce reverse mutation in the parental strain, was added to this culture. The culture was incubated for one hour to induce reverse mutation in mutants sensitive to NG. Then the cells were spread onto a lysine-lacking M9 minimal agar plate which contained 0.6% Na₂HPO₄, 0.3% KH₂PO₄, 0.1% NH₄Cl, 0.02% MgSO₄·7H₂O, 0.2% glucose, 1 µg/ml of vitamin B1 hydrochloride and 50 µg/ml each of leucine and tryptophan, and incubated at 37°C for 2 days. The *lys*⁺ revertant colonies which appeared on these plates would be produced by the *lys*⁺ reverse mutation (i) induced by the initial NG treatment, (ii) induced by a small amount of NG in the second treatment, (iii) induced spontaneously. To distinguish them, the *lys*⁺ revertants were replicated onto a leucine-lacking M9 minimal agar plate with or without a small amount of NG. The cells which formed colonies on the plate with NG but not without NG could be the mutants which we were looking for. The degree of NG-sensitivity of the mutants selected was determined by a reversion test in which a paper disk containing a small amount of NG was placed on the leucine-lacking M9 minimal agar plate with a lawn of each mutant to test. By this procedure, we could isolate 11 mutants, which showed high reversion in response to a low level of NG, from about 10⁷ cells (about 3% of the *lys*⁺ revertants). For example, strains MS10 and MS47 gave rise to *leu*⁺ revertants with high frequency at 0.2 µg of NG per ml, of which concentration was not high enough to make the parental strain revert at significant frequency. Interestingly, they did not show high sensitivity to mutagens other than NG as shown in Table I. Thus these mutants showed preferentially high sensitivity to NG-mutagenesis.

Since the frequency of appearance of NG-sensitive mutants was found to be approximately 10⁻⁶, the method described proved to be quite efficient for selecting such mutants. Moreover, the mutants speci-

TABLE I. RELATIVE VALUES^{a)} OF SPONTANEOUS AND MUTAGEN-INDUCED *leu*⁺ MUTATION FREQUENCIES IN MUTANTS AND THE PARENTAL STRAIN

Strain	Spontaneous	Mutagen-induced							
		NG (µg/ml)		Ethylmethane-sulfonate (%)		Methylmethane-sulfonate (%)		4-Nitroquinoline 1-oxide (µM)	
		0.2	1.0	0.1	1.0	0.05	0.1	1.0	100
W3876LTL	1 (7.3) ^{b)}	1 (8.2)	1 (184)	1 (11)	1 (122)	1 (25)	1 (46.5)	1 (7.4)	1 (42.1)
MS10	0.84	54.2	12.1	2.1	1.0	2.0	1.4	1.5	1.4
MS47	1.2	86.1	19.4	0.9	0.7	3.9	3.2	1.2	1.0
W3876LT <i>LuvrA</i> ^{c)}	1.6	ND ^{d)}	1.1	ND	8.3	ND	1.2	ND	20.4

^{a)} Relative value is the ratio of a reverse mutation frequency of the mutants to that of strain W3876LTL.

^{b)} Figures in parentheses indicate numbers of *leu*⁺ revertants per 10⁸ survivals.

^{c)} Strain W3876LT*LuvrA* was derived from W3876LTL by Pl-transduction, in which strain H5 17-9 was used as a donor.

^{d)} ND: not determined.

fically sensitive to NG appear to be valuable for better understanding of the mechanism of NG-mutagenesis. The genetic analysis of the mutants is now in progress.

REFERENCES

- 1) B. N. Ames, "Chemical Mutagen: Principles and Methods for their Detection," Vol. 1, ed. by A. Hollaender, Plenum New York, 1971, p. 267.
 - 2) J. McCann, N. E. Spingarn, J. Kabori and B. N. Ames, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 979 (1975).
 - 3) B. N. Ames, F. D. Lee and W. E. Durston, *Proc. Natl. Acad. Sci. U. S. A.*, **70**, 782 (1973).
 - 4) E. E. Slater, M. D. Anderson and H. S. Rosenkranz, *Cancer Res.*, **31**, 970 (1971).
 - 5) T. Kada, Y. Sadaie and K. Tutikawa, *Mutat. Res.*, **16**, 165 (1972).
 - 6) M. H. L. Green and W. J. Muriel, *ibid.*, **38**, 3 (1976).
 - 7) E. A. Adelberg, M. Mandel and G. C. G. Chen, *Biochem. Biophys. Res. Commun.*, **18**, 788 (1965).
-