TEST STRAINS OF *Escherichia coli* FOR THE DETECTION OF CHEMICAL MUTAGENS

A. P. Pekhov and V. N. Reshetnikova

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After treatment of strain *Escherichia coli* AB2500 with acridine orange and 5-bromouracil two temperature-sensitive mutants were isolated: AP 16 and AP 18. These mutants were found to be sensitive and they formed revertants on treatment with N-methyl-N'-nitro-N-nitrosoguanidine, hydroxylamine, nitrous acid, sodium metabisulfite, methyl methanesulfonate, and proflavine. The introduction of an additional mutation into these strains, causing an increase in their sensitivity to crystal violet, caused some increase in their ability to form revertants through the action of proflavine and methyl methanesulfonate.

KEY WORDS: *mutant; mutagen; mutagenic activity; test strain; test system.*

The sharp increase in the number of newly synthesized chemical compounds and the prospects of their use in different branches of experimental biology and medicine have raised the problem of the determination of the mutagenic activity of these compounds, for many of them, as the result of molecular-genetic investigations have shown, are capable of inducing mutations [1-3]. In order to discover chemical mutagens, several test systems have been developed in recent years in which organisms belonging to different species have been used as test objects (bacteria, yeast, insects, animals) [4-7]. However, despite definite progress in the creation of such test systems, a general disadvantage of them is that they are either insufficiently sensitive or they are not universal, in the sense of detecting mutagens with different mechanisms of action.

In this investigation an attempt was made to create a simple bacterial test system characterized not only by efficiency, but also by its ability to determine the mutagenic activity of chemical compounds inducing mutations consisting both of replacement of the nitrogenous bases in DNA and mutations of the "frame shift" type.

EXPERIMENTAL METHOD

Strain *Escherichia coli* AB2500 (thr^- leu^- pro^- his^- arg^- thi^- str^r drm tsx sup37 uvr A^- thy^-), a derivative of strain *E. coli* AB1157, was used as the original strain.

To induce a temperature-sensitive (ts) mutation, cells of this strain were treated with acridine orange and 5-bromouracil in concentrations of 50 and 300 μg/ml, respectively. The ts mutants were isolated by incubating seedings of cells treated with mutagen at 44-45°C.

To induce mutation accompanied by an increase in sensitivity of the ts mutants to crystal violet, these mutants were treated with nitrosoguanidine. The search for mutants sensitive to crystal violet was conducted on meat–peptone agar (MPA) containing this dye in concentrations of 100 μg/ml.

The sensitivity and ability of the isolated mutant to form revertants were tested by treating them with classical mutagens: N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), hydroxylamine (HA), nitrous acid (HNO_2), sodium metabisulfite, methyl methanesulfonate (MMS), and

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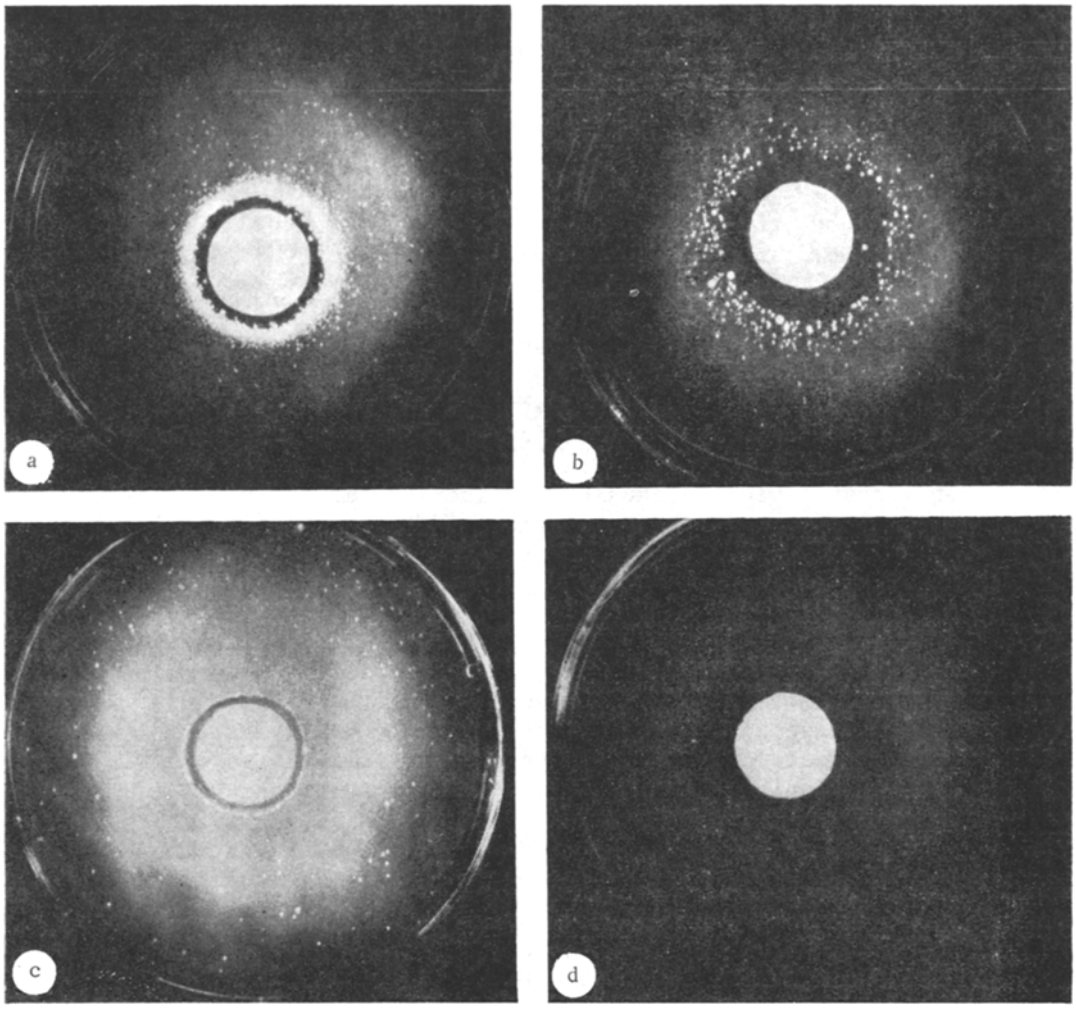

Fig. 1. Sensitivity and revertant forming ability of strain AP 16 after treatment with MNNG (a) and HA (b) and of strain AP 16-3 after treatment with proflavine (c); control (d).

proflavine in the "spot test" suggested by Ames [1]. For this purpose, 18-h cultures of mutant [in meat–peptone broth (MPB) + thymine in a concentration of 2 μg/ml] were cultured in a volume of 0.5 ml on MPA + thymine. A disk of filter paper, impregnated with a solution of one of the mutagens, was placed on the lawn of seeded bacteria. The dishes were incubated for 40 min at 34°C, then for 24 h at 44-45°C and, finally, for 1-2 days at 34°C.

Solutions of the mutagens were made up just before use: MNNG in phosphate buffer, pH 7.2, HA in physiological saline, pH 6.0, MMS in 0.2 M Tris buffer, pH 7.5, sodium metabisulfite in 0.1 M acetate buffer, pH 5.2, and proflavine in 0.2 M Tris buffer, pH 10.0. Nitrous acid was prepared by dissolving sodium nitrite in 0.1 M acetate buffer, pH 4.6.

EXPERIMENTAL RESULTS

The search for ts mutants of strain *E. coli* AB2500 was carried out, as mentioned above, after treatment with acridine orange and 5-bromouracil. In all the experiments 28,092 colonies were arising from cells treated with acridine orange and 15,434 colonies from cells treated with 5-bromouracil were analyzed.

As a result of these investigations temperature-sensitive mutants AP 16 (from cultures treated with acridine orange) and AP 18 (from cultures treated with 5-bromouracil) were isolated. These mutants were characterized by loss of ability to grow on NA at 44-45°C.

The ts mutants were tested for their sensitivity and their ability to form revertants when treated with classical mutagens in experiments in which a disk of filter paper, soaked in a solution of one of the mutagens, was placed on a lawn of seeded bacteria ($1 \cdot 10^8$–$2 \cdot 10^8$

cells/ml); the solutions of mutagens were made up in the following concentrations: MNNG 2 mg/ml, proflavine 2 mg/ml, HA 0.5 M, sodium metabisulfite 0.5 M, MMS 0.2 M, HNO_2 2 M. To impregnate one disk not more than 0.05-0.1 ml of the original mutagen was needed.

Strain AP 16 proved to be sensitive to MNNG, HA, sodium metabisulfite, and MMS; around disks impregnated with the mutagens a zone of inhibition of cell growth (a zone of lysis) appeared, with revertant colonies visible around its edge, i.e., colonies which had acquired ability to grow on MPA at 44-45°C. The results of experiments obtained with strain AP 16 treated with MNNG and HA are given in Fig. 1.

A similar pattern was observed with strain AP 18, when treated with MNNG, HA, sodium metabisulfite, and proflavine.

Strain AP 16 was insensitive but it formed revertants when treated with HNO_2 and proflavine, as did strain AP 18 when treated with HNO_2 and MMS.

After treatment of cultures of strains AP 16 and AP 18 with nitrosoguanidine, in the first case 3400 colonies and in the second case 3300 colonies were analyzed; mutants AP 16-3 and AP 18-10 sensitive to crystal violet were selected from them.

Introduction of the additional mutation did not change the sensitivity of strain AP 16-3 to MNNG, HA, sodium metabisulfite, MMS, and HNO_2, but it increased, although only slightly, its sensitivity to proflavine (Fig. 1). Strain AP 18-10 proved to be more sensitive to MMS.

It can be concluded from the results that, unlike other known test systems, the cells of the strains now isolated are sensitive simultaneously to mutagens differing in their mechanism of action. On the one hand, they are sensitive to mutagens inducing replacement of bases (N-methyl-N'-nitro-N-nitrosoguanidine, hydroxylamine, methyl methanesulfonate, sodium metabisulfite, and HNO_2), and on the other hand, to mutagens inducing mutations of the "frame shift" type (proflavine). Accordingly, the models of the strains of bacteria thus developed are promising as test systems for verifying the mutagenic activity of chemical compounds.

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