# MUTAGENS AND ANTIMUTAGENS IN FOOD

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ABSTRACT

Man is probably most closely connected with the environment by ingesting food into the body. Since mutagens are thought to cause genetic disease, cancer, aging, etc., those in food must be most carefully assessed. In addition, we recently developed studies on food antimutagens which inactivate mutagens or suppress cellular mutagenesis.

1. Detection of food mutagens and carcinogens by the *Bacillus Bacillus subtilis* rec-assay.
   Chemicals which inhibit the growth of recombination repair-deficient cells more than that of wild cells are considered to possess DNA-damaging characters. In the past decade, we have been involved in several domestic and international projects aimed at evaluating shortterm assays for detecting mutagens and carcinogens. It was concluded that the *Bacillus subtilis* rec-assay is one of the most efficient and economical procedures.
2. A detection and assessment of food mutagens by the *Salmonella* SD-assay
   Mutations from streptomycin (SM)-dependency to SM-independency were adopted in order to detect mutagens in food containing histidine which can not be carried out with histidine-reversion assays. Mutation-induction capabilities using one 9 of many kinds of food were quantitatively compared.
3. Studies on food factors suppressing the mutagenicity
   a. Desmutagen
      Food factors that inactivate mutagens *in vitro* were studied. We will especially discuss in detail the vegetable desmutagens.
   b. Bio-antimutagens
      Food factors that suppress cellular mutagenesis were studied. We will especially discuss in detail antimutagenic characters of cinnamon and green-tea factors.

## INTRODUCTION

Man is probably most closely connected with the environment by ingesting food into the body. Since mutagene are thought to cause genetic disease, cancer, aging, etc., those in food must be most carefully assessed. Foods contain mutagens that can be classified as follows:

(a) Mutagens added as food additives.
(b) Contaminants from the environment.
(c) Those produced in the course of processing or cooking of food.
(d) Natural mutagens existing as ingredients of food.

In any case, our foods are characterized in each country by climate, economics, style or habit of life, etc. Therefore, we have to check the safety of food from the standpoint of regional specificities.

Detecting mutagens in food is the first step. We should then find out how to prevent them. We recently developed studies on food antimutagens which inactivate mutagens or suppress cellular mutagenesis.

## FOOD MUTAGENS: EXPERIENCES IN JAPAN

Studies on the environmental mutagens started around 1970 in Japan. Prof. Tazima, former director of the National Institute of Genetics, formed a group of genetists. We decided first to check the mutagenicity of food additives that have been used in Japan. Prof. Tonomura proposed studying nitrofuran derivatives. One of them, AF2, a food-preservative widely used, was found to be extremely positive in the *Bacillus subtilis* rec-assay and *E. coli* reversion assays. We proposed suspension of the use of AF2 to the Ministry of Welfare[1].

The Ministry of Health and Welfare then promoted a 5 year project in which about 30 laboratories of different specialities collaborated in evaluation of the short-term assays to detect and to predict carcinogenicity and genotoxicity of chemicals including food-additives, pesticides, cosmetics, etc. Results and conclusions obtained are summarized in Table 1[2].

As the mutagenicity assays have improved their sensitivities and specificities, new mutagens of various types have become known on a world-wide scale. Their evaluation and prevention are subjects that are actively studied and discussed. They include:

(a) Plant ingredients such as flavonoids.
(b) Pyrolysist products from proteins and amino-acids such as Trp-P-1, Glu-P-1, IQ, etc.
(c) Nitro-arenes produced by nitrosation of polycyclic hydrocarbons, such as pyrene, etc.

## DETECTION OF FOOD MUTAGENS AND CARCINOGENS BY THE *Bacillus subtilis* REC-ASSAY[3–6]

Chemicals which inhibit the growth of recombination repair-deficient cells more than that of wild cells are considered to possess DNA-damaging characters. In the past decade, we have been involved in several domestic and international projects aimed at evaluating short-term assays for detecting mutagens and carcinogens[2, 7, 8]. It is now acknowledged that the *Bacillus subtilis* rec-assay in one of the most efficient and economical procedures.

The simplest assay procedure is to use spores. Two suspensions of vegetative cells or spores of strains H17 $Rec^+$ and M45 $Rec^-$ are streaked radially on the "dry" surface of both agar. A sample solution is placed on a paper disk and then placed on the starting point of the streaks by incubating the plate at 37°C overnight and the results are obtained (Fig. 1).

Table 1. Summary of short-term assays for carcinogenicity.

| Mutagenicity | Carcinogenicity | No. (%)[a] of Compounds | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Salmonella | Rec assay | Chromosomes | | Salmonella + rec assay + chromosomes | Salmonella + chromosomes | Whole animal |
| | | | | In vitro | In vivo | | | |
| + | + | 33(67%) | 19(43%) | 23(58%) | 19(68%) | 41(52%) | 39(61%) | 42(52%) |
| + | – | 16 | 25 | 16 | 9 | 38 | 25 | 39 |
| – | – | 48(73%) | 31(63%) | 30(77%) | 34(54%) | 31(79%) | 43(81%) | 30(81%) |
| – | + | 18 | 18 | 9 | 28 | 8 | 10 | 7 |
| + | ? | 14 | 16 | 26 | 9 | 33 | 34 | 31 |
| – | ? | 53 | 40 | 32 | 44 | 31 | 24 | 30 |
| | | 182 | 149 | 136 | 143 | 182 | 182 | 182 |

[a] Percentage of total positive of negative for mutagenicity.

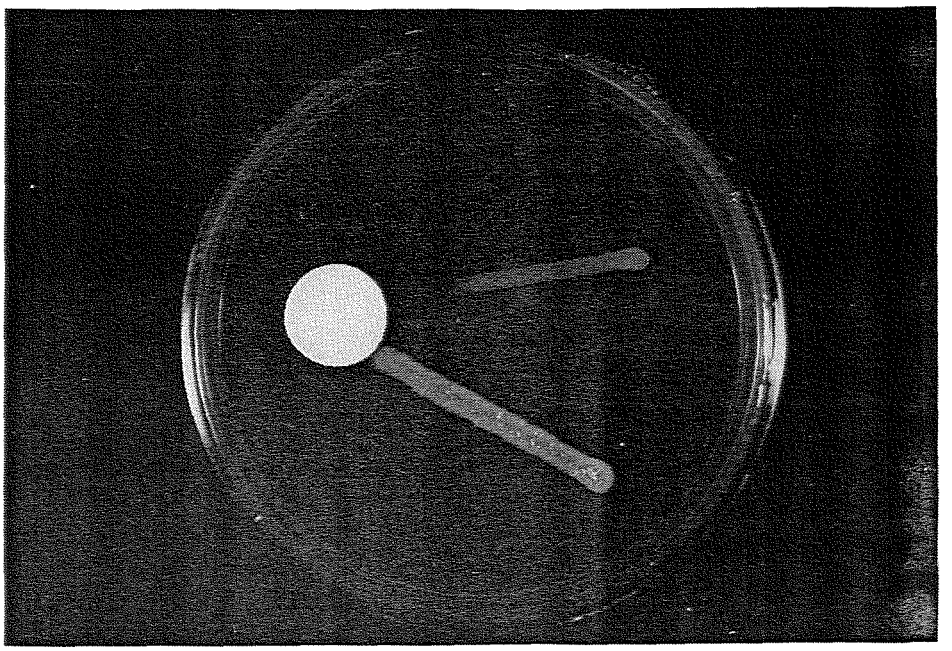

Fig. 1. A typical rec-assay positive plate.

It is highly recommended that an equipped microbiology laboratory prepare the spores and distribute them to different laboratories. Spores are quite stable for more than 2 years if stocked at +4°C. Whoever would like to obtaine authentic spores should write to the author Dr. T. Kada, National Institute of Genetics, Mishima, Shizuoka-ken 411, Japan.

For mutagens requiring metabolic activation for their activities, the spore-S9 agar method[5] is recommended (Fig. 2). Quantitative aspects of the rec-assay are discussed elsewhere[6].

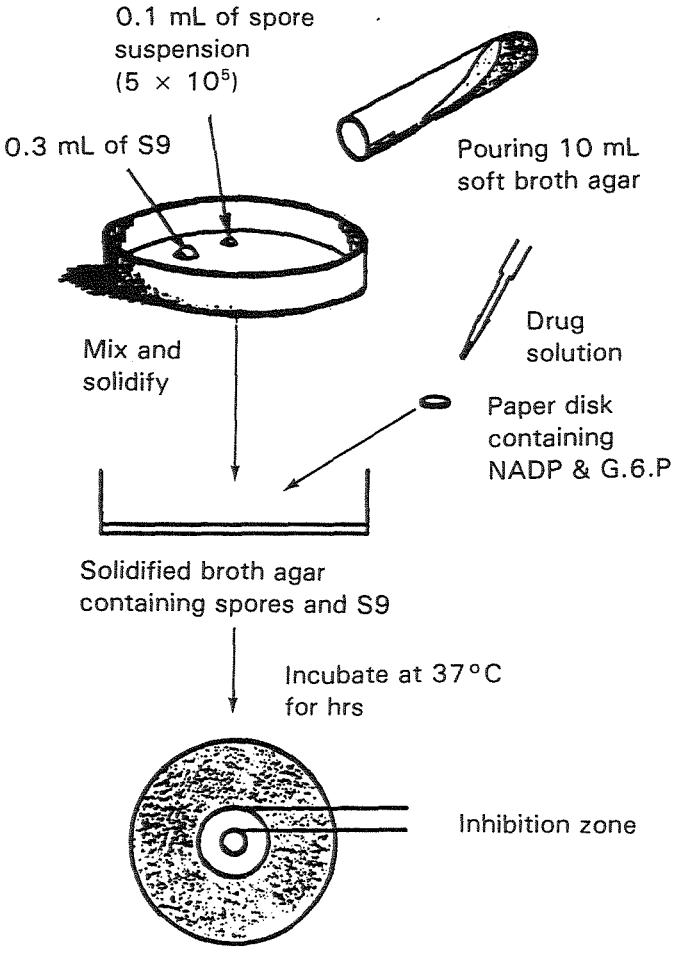


Fig. 2. Procedure of spore rec-assay.

## A DETECTION AND ASSESSMENT OF FOOD MUTAGENS BY THE *Salmonella* SD-ASSAY[9]

Mutation from streptomycin (SM)-dependency to SM-independency were adopted in order to detect mutagens in samples such as food containing histidine which interfere with histidine-reversion assays[10]. *The simplest assay procedure* is as follows:

The SM-d *Salmonella* strain is grown 40 hours in liquid broth containing 20 $\mu$g/ml of streptomycin and diluted $10^{-1}$ with SM-free broth. 0.1 ml portion is spread on the surface of broth agar in which the sterile sample is

incorporated. After incubation at 37°C for 3 days, the number of SM-independent colonies is counted. Results of a red-wine sample are shown in Fig. 3.

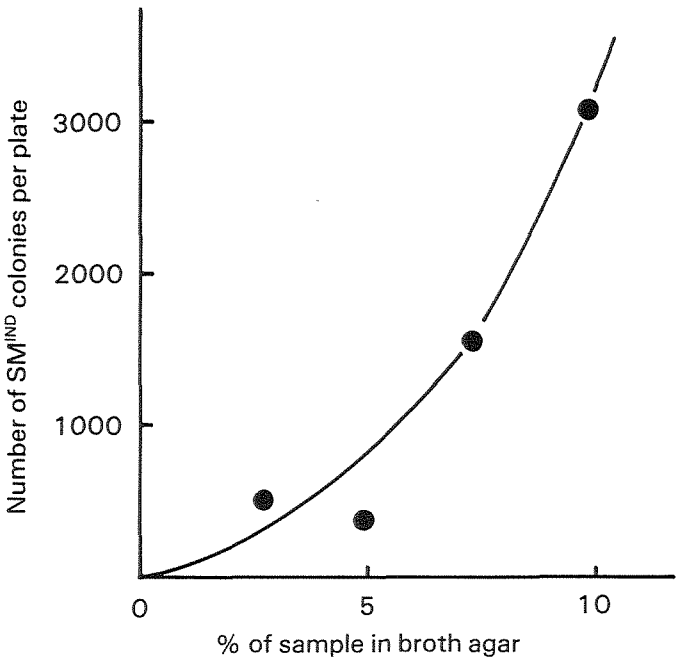


Fig. 3. Induction of $SM^d \rightarrow SM^{ind}$ mutations by a wine sample. The sample was sterilized by filtration though a Millipore filter and incorporated into broth agar. Then cells of SD100 were spread on the agar and incubated at 37°C for three days.

We carried out the SD-assays with a number of foodstuffs and mutation-induction capabilities of one g each of the samples were quantitatively compared (Table 2).

Table 2. Certain examples of the mutagenic potency per g (mut/g) of food samples. The mut/g values indicate that one gram (or milliliter) of the samples gives this number of revertants in each standard mutagenicity assay.

| | |
|---|---|
| Drinking water (Based on a report from Canada | 0.047 |
| Food contaminated with aflatoxin $B_1$ at 1 ppm | 12,000 |
| Broiled beef (Containing 53 ng of Trp-P-1) | 2,067 |

## STUDIES ON FOOD FACTORS SUPPRESSING THE MUTAGENICITY

### Definition of Desmutagens and Antimutagens

Research on antimutagens in microorganisms, which started around 1959, is reviewed by Clarke and Shankel[11]. In these studies, antimutagens are considered to be acting primarily on the level of the cell. Recently, modification factors affecting the yield of mutations induced by many chemicals mutagens have been discovered. Some factors are acting directly on mutagens without affecting the cell. We have used the term "desmutagen" — coined by Dr. John Drake of Research Triangle, North Carolina — in studies where mutations induced by tryptophan pyrolysates and other substances were shown to be suppressed by vegetable factors[12, 13]. These factors were directly inactivating the mutagens.

On the other hand, the term "antimutagen" was originally defined as an agent that reduces the apparent yield of spontaneous and/or induced mutations, regardless of the mechanisms involved. Therefore the original "antimutagens" include "desmutagens". However, in this paper we will define "antimutagens" as factors acting solely on the cellular level for the suppression of mutations. These ideas are shown schematically in Figure 4[14, 15].

### Desmutagens

Since mutagens are by nature reactive with DNA, they can react with other cellular components, such as proteins. A typical example is the pesticide captan, which is easily inactivated by cellular SH agents[16]. Consequently the carcinogenicity of captan is very weak or absent in spite of its very high mutagenecity. Takahashi and his collaborators[17] systematically studied the desmutagenic factors of typical mutagens (Table 3) by means of the *Bacillus subtilis* rec-assay[3–6].

Much attention has been given to mutagenic products in charred protein (see for review; Sugimura *et al.*)[18]. We looked for desmutagenic agents in food, and found that extracts from certain vegetables worked as desmutagens with mutagenic pyrolysis products originating from tryptophan[12, 13]. Results of *Salmonella* reversion assays are shown in Figure 5, where cabbage extracts considerably depress the mutagenicity of the tryptophan pyrolysates[19]. When pyrolysis products from several amino acids were treated with juices obtained from different vegetables, it was found that the juices of eggplant, burdock, and broccoli have wider spectra of inactivation then other juices[13] as shown in Figure 6.

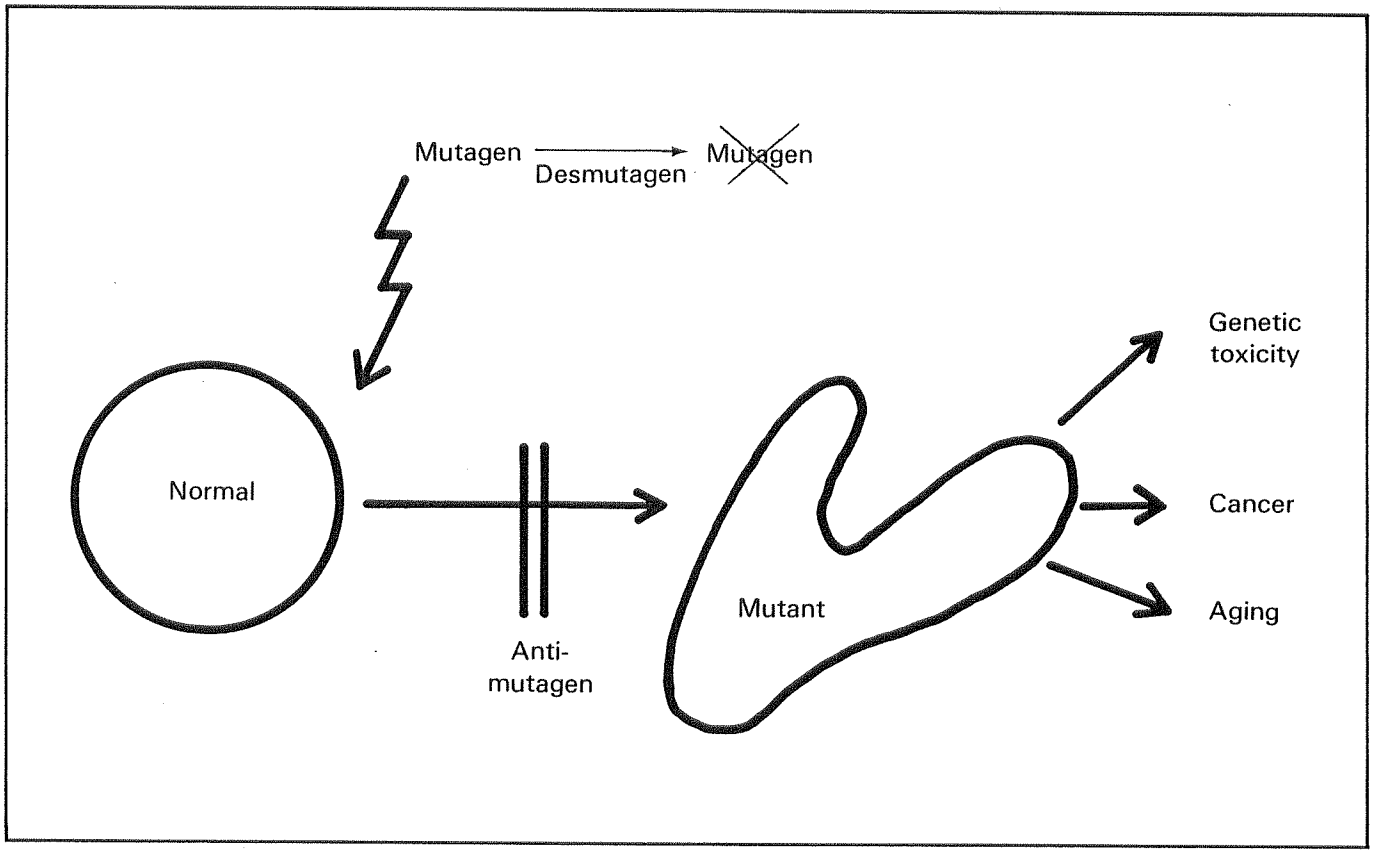


Fig. 4. Schematic actions of desmutagens and antimutagens.

Table 3. Desmutagens for typical mutagens.

| Test Agent | Addition ( $\mu$ mol) | Mutagen ( $\mu$ mol) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Dexon 0.04 | Captan 0.03 | MMC $8 \times 10^{-5}$ | Doxorubicin.HCl 0.18 | AF2 $1 \times 10^{-3}$ | Formaldehyde 33 | 4NQO $3 \times 10^{-5}$ | MNNG 0.34 |
| Water | | − | − | − | − | − | − | − | − |
| DMSO | | − | − | − | − | − | − | − | − |
| L·AsA | 1.0 | + + | + + | + + | − | − | − | − | + + |
| D·EA | 1.0 | + + | + + | + + | − | − | − | − | + + |
| BHT | 1.0 | ± | + | − | − | − | − | ± | − |
| GA | 1.0 | − | ± | + + | − | − | − | − | − |
| PG | 1.0 | + + | ± | + | − | − | | − | − |
| VE | 1.0 | − | + | − | | | | | |
| Dithionite | 0.2 | − | + + | + + | − | − | | − | − |
| GSH | 1.0 | − | + + | + + | − | − | | − | − |
| L-cysteine | 1.0 | + + | + + | + + | + + | + + | − | + | + + |
| L-cystine | 1.0 | + + | + + | + + | + + | + + | = | + | + + |
| Cysteamine | 1.0 | + | + + | + | − | − | | + | + + |
| Histamine | 1.0 | − | − | − | − | − | | − | − |
| Thiourea | 1.0 | − | + + | − | − | − | | − | − |
| AET | 1.0 | − | + + | + + | + + | − | | − | − |
| S9 fraction | 50 $\mu$ l | − | + + | + | ± | − | + | + + | + |
| Albumin | 500 $\mu$ l | − | + | − | − | − | − | ± | − |

Note: The *B. subtilis* rec-assays were carried out. + +, Strongly desmutagenic;, Weakly desmutagenic; −, Not desmutagenic.
Source: Onitsuka *et al.* (1978).

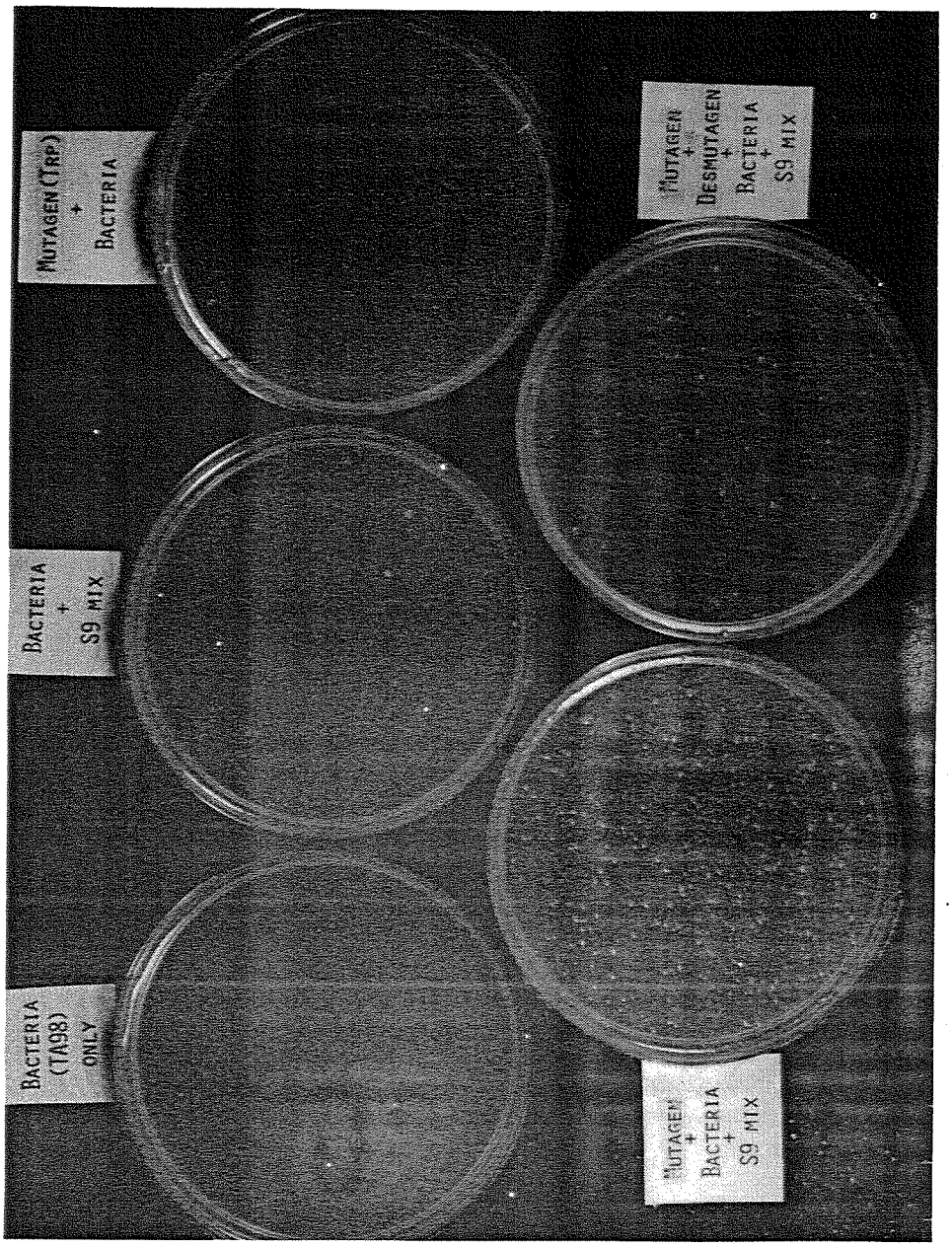


Fig. 5. Desmutagenic effects of the cabbage extract on the mutagenicity of tryptophan pyrolysates. *Salmonella* reversion assays were carried out using the strain TA98 with standard rat liver homogenate (S9).


Fig. 6. Desmutagenic activities of extracts from ten foodstuffs on the mutagenicity of pyrolysis products from five amino acids. The standard assays (see the original text) gave the following numbers of $His^+$ revertants per plate: for L-citruline, 154/1000 $\mu$g, L-lysine, 143/1000 $\mu$g; L-ornithine, 384/1000 $\mu$g, L-arginine, 262/400 $\mu$g; L-tryptophan, 357/100 $\mu$g. Inactivated parts of each mutagenic amino acid pyrolysis product by each food extract are shown in percentage (Morita *et al.*, 1978).

## Bio-Antimutagens

Among the biometal compounds screened, we found that the frequencies of mutations induced by NTG (N-methyl-N-nitro-N-nitrosoguanidine) or by γ-rays in *Escherichia coli* B/r WP2 *trp* were reduced markedly by the presence of cobaltous chloride ($CoCl_2$) in the post-treatment medium of these bacteria[20, 21], as shown in Table 4 and Figure 7. It has been shown that the frequency of mutations toward 8-azaguanine resistance was also significantly reduced by the presence of cobaltous chloride of 1 μg/ml during the expression period of *in vitro* cultured cells of Chinese hamster (Yokoiyama, personal communication).

Table 4. Effects of cobalt chloride on lethality and mutation induction in *E. coli* B/r WP2 *try*⁻ cells previously treated or not treated with MNNG (60 μg/ml for 30 mins).

| Concentration of $CoCl_2.6H_2O$ (μg/ml) in MB agar | Cells not treated with MNNG | | | Cells treated with MNNG | | |
|---|---|---|---|---|---|---|
| | Mean number of try⁺ colonies per plate | Number per ml of viable cells | Frequency of try⁺ revertants per $10^8$ cells | Mean number of try⁺ colonies per plate | Number per ml of viable cells | Frequency of try revertants per $10^8$ cells |
| 0 | 5.7 | $4.0 \times 10^9$ | <1.4 | 2,335 | $3.4 \times 10^9$ | 686.9 |
| 5 | 3.0 | $4.5 \times 10^9$ | <0.7 | 2,243 | $3.5 \times 10^9$ | 640.8 |
| 10 | 0.7 | $4.8 \times 10^9$ | <0.2 | 453 | $3.0 \times 10^9$ | 151.0 |
| 15 | 1.0 | $2.9 \times 10^9$ | <0.4 | 79.7 | $2.9 \times 10^9$ | 27.5 |
| 20 | 0.3 | $3.7 \times 10^9$ | <0.1 | 20.0 | $2.0 \times 10^9$ | 10.0 |

Food factors that suppress cellular mutagenesis were also shown. We are especially studying in detail anti-mutagenic characteristics of cinnamon and green-tea factors[22–24].

In conclusion, we have actually practical means to assess mutagenicities and antimutagenicities of food. These aspects may be quite dependent on habits of life in each country or region. Continued surveys are necessary for people's health.

I thank very much Dr. A. Matsuyama for his advice and encouragement in this study.

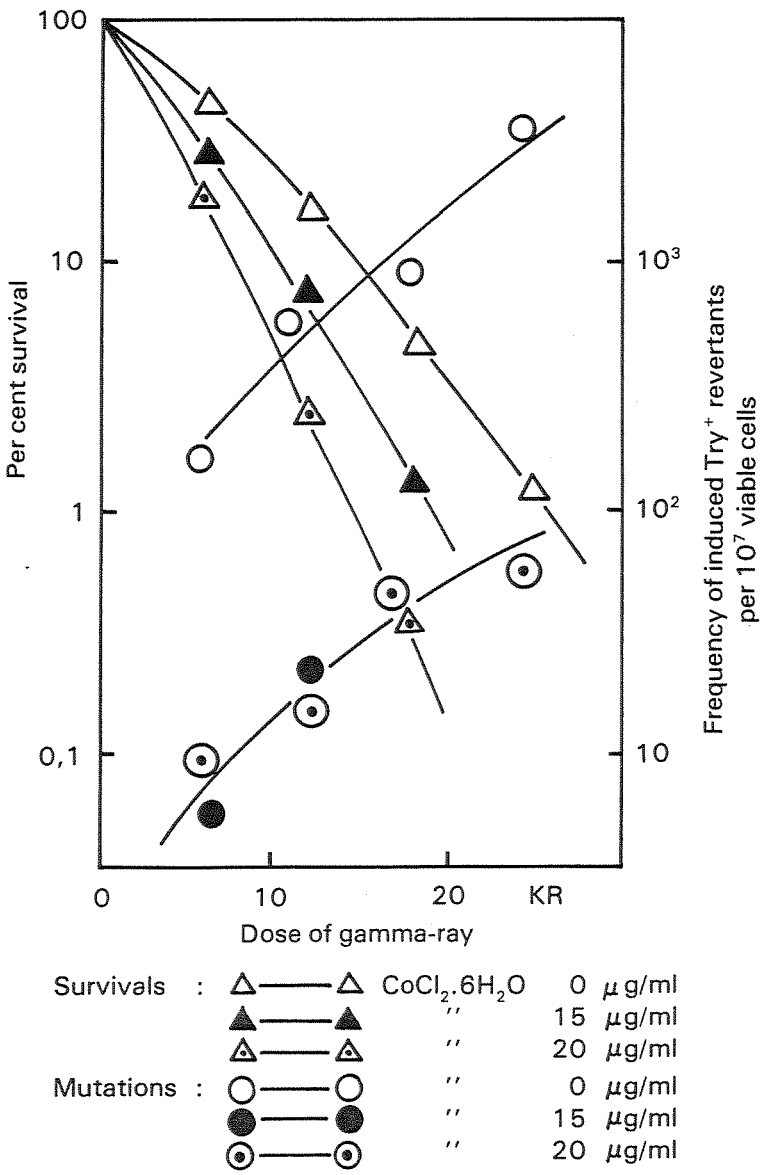


Fig. 7. Effect of cobaltous chloride on γ-ray induction of mutations in *Escherichia coli* WP2 B/r *trp*. Bacteria grown over-night in broth were washed, exposed to γ-ray from a $^{137}Cs$ source at 0°C and plated after different dilutions on semi-enriched minimal agar containing cobaltous chloride and incubated for 2 days at 37°C.

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