Prophage Induction in *Escherichia coli* (Lambda) by
*N*-Nitrosamines

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Carcinogenic *N*-nitrosamines were tested for their ability to induce \( \lambda \) in a lysogenic strain of *Escherichia coli* K-12 (58-161 F\(^+\)). Dimethylnitrosamine, di-\( n \)-propylnitrosamine, methyl-\( n \)-propylnitrosamine, and *N*-nitrosopiperidine were shown to be inducers of prophage.

An ever-increasing wealth of evidence supports the correlation between the ability of a substance to induce lysogenic bacteria and its carcinogenic activities (2, 4, 5). Although not all carcinogens or antineoplastic agents tested have been shown to be inducers (9), the correlation is such that induction can be used as a rapid in vitro screening system for potentially harmful carcinogens and useful antineoplastic compounds (3). Many *N*-nitrosocompounds have been shown to be both carcinogens and inducers. However, *N*-nitrosamines, which are among the most potent carcinogens known (7), have not been shown to have inducing abilities (4).

The present study was undertaken in an attempt to establish an induction system sensitive to *N*-nitrosamines.

*Escherichia coli* 58-161, lysogenic for \( \lambda \), and *E. coli* C600, sensitive to \( \lambda \) and resistant to streptomycin, were used in the induction experiments. Dimethylnitrosamine, di-\( n \)-propylnitrosamine, methyl-\( n \)-propylnitrosamine and *N*-nitrosopiperidine, and diethylamine (used as a control) were dissolved in distilled water. For \( \lambda \) induction, a modification of the method of Price et al. (9) was used. Overnight cultures of 58-161 in *E. coli* minimal medium and 0.2% Casamino Acids were diluted 1:10 in fresh medium and aerated at 37°C for 1 h. Cells were resuspended in fresh medium to a concentration of \( 3 \times 10^8 \) cells/ml. To 0.8 ml of this suspension was added 0.2 ml of the inducing agent, and the mixture was incubated at 37°C for 60 min. After this induction period, 9 ml of prewarmed heart infusion broth and 0.1% glucose were added, and incubation was carried out for 2 h in a 37°C water bath with reciprocal agitation. Samples were diluted in 0.85% NaCl and tryptone broth (Difco tryptone, 13 g; NaCl, 8.5 g; glucose, 1.5 g; distilled water, 1 liter) and plated for plaque formers. The remainder of each test sample was incubated further for 8 h to permit the development of detectable turbidity. Test samples failing to show growth were retested at lower nontoxic concentrations. Controls of 58-161 without inducer were carried out for each experiment. If the level of spontaneous induction was greater than \( 10^2 \) plaque-forming units/ml, the experiment was disregarded. Positive controls with 5 to 6 \( \mu \)g of mitomycin C per ml, a known inducer, were also included in each experiment.

To enumerate plaque formers, the indicator strain C600 was grown in heart infusion broth and 0.1% glucose and used to seed the top agar layer of soft nutrient agar (0.5% agar) supplemented with 0.5% NaCl. The base agar layer was tryptone agar (tryptone broth and 1.5% agar) containing 100 \( \mu \)g of streptomycin per ml. Plaques were counted after incubation for 48 h. Samples which gave an induction index of 10 (10 times the spontaneous plaque count) or greater were considered to be active inducers of the lytic cycle of 58-161 cells (5).

The concentrations of nitrosamines at which induction was obtained are shown in Table 1. No induction was obtained with diethylamine. Thus, the ability of the carcinogenic *N*-nitrosamines tested to induce \( \lambda \) in a lysogenic strain of *E. coli* provides a screening system for potentially harmful nitrosamines. In the system outlined above the minimal concentrations of inducers are similar to those for *N*-nitrosamidines and *N*-nitrosamides which range from 0.1 to 25 \( \mu \)g/ml (4).

As prophage induction involves an alteration of the bacterial nucleic acid balance in which deoxyribonucleic acid synthesis is selectively blocked (6) and as many carcinogens bind specifically to deoxyribonucleic acid (8), this binding may be the cause of the observed
TABLE 1. *E. coli* (λ) induction by nitrosamines

| Inducing agent                  | Lowest effective level of N-nitrosamine (µg/ml) |
|---------------------------------|-----------------------------------------------|
| Dimethylnitrosamine             | 0.2                                           |
| Di-n-propylnitrosamine          | 0.14                                          |
| Methyl-n-propylnitrosamine      | 0.17                                          |
| Nitrosopiperidine               | 0.17                                          |
| Diethylamine                    | No induction                                  |

It is thought that nitrosamine carcinogenicity may depend on the presence of at least one disposable hydrogen atom in the α position (1), and the significance of this in relation to the process of prophage induction should be investigated.

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