Mutagenic Action of N-Methyl-N'-Nitro-N-Nitrosoguanidine on Gaffkya tibisci at Alkaline pH

MENSURA DRAŽIĆ AND VLADIMIR DELIĆ
Research Department, "PLIVA" Pharmaceutical and Chemical Works, L. Ribara 89, Zagreb, Yugoslavia

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Good yields of mutants were obtained by incubating cells of Gaffkya tibisci with N-methyl-N'-nitro-N-nitrosoguanidine in alkaline buffer. Nearly 50% of the survivors were auxotrophic mutants under the conditions described.

N-methyl-N'-nitro-N-nitrosoguanidine (NTG) has been widely used to induce mutations in microorganisms. Adelberg, Mandel, and Chen (1) found that up to 42.5% of the survivors of NTG treatment were auxotrophic mutants in Escherichia coli, whereas in some other microorganisms lower frequencies were found; in both Salmonella typhimurium (6) and Schizosaccharomyces pombe (8), about 8% auxotrophs were reported.

Recently Delić, Hopwood, and Friend (4) found that mutagenesis by NTG in streptomycetes is quantitatively related to the decomposition of NTG in alkaline buffers. Since the highest auxotrophic mutant yields [22% in Streptomyces rimosus ATCC 10970; 12% in Streptomyces coelicolor A3 (2)] were obtained at pH 9 (4), the purpose of the experiments reported here was to find whether this phenomenon is restricted to streptomycetes or whether mutagenesis by NTG is also higher at alkaline pH in a eubacterium of practical interest.

Gaffkya tibisci is a producer of L-glutamic acid by fermentation. The wild-type strain isolated from soil (7) is prototrophic. Some auxotrophic mutants were previously isolated after ultraviolet mutagenesis (5). Several of these auxotrophs were tested for reversion by NTG by a plate test (2). One of them, an arginine-requiring (Arg-) mutant, showed a clear ring of prototrophic colonies induced by NTG (Fig. 1) and was chosen for experiments on the pH dependence of mutagenesis.

The Arg- strain was grown in nutrient broth (Difco). Exponential-phase cultures had a final density of $2 \times 10^8$ to $5 \times 10^8$ cells per ml. Cultures were centrifuged for 10 min at $3,000 \times g$ and washed twice with saline. Pellets were suspended in 1 ml of tris(hydroxymethyl)amino-methane-maleic acid (TM) buffer, in which TM replaced the phosphate salts in the minimal medium of Davis and Mingioli (1, 3). NTG was dissolved in cold (4 C) TM buffer at appropriate concentrations. One volume of suspension was transferred to the appropriate volume of NTG solution, and the solution was agitated and incubated in a water bath at 37 C. Samples (3 ml) were withdrawn at different periods of time, filtered on 47-mm membrane filters (HA type, 0.45-μm pore size; Millipore Corp., Bedford, Mass.), and washed twice with saline. Filters were transferred to 3 ml of water and agitated, and appropriate dilutions of the suspensions were plated on minimal medium (3) for auxotrophy reversions, and on nutrient agar for survivors. Plates were incubated at 37 C for 4 days, and colonies of induced revertants and survivors were counted.

In Fig. 2, the pH dependence of the proportion of Arg+ revertants among survivors in a standard time of treatment is presented. It is obvious that Arg+ reversion frequencies increased with the pH of the buffer up to at least pH 9. Mutant frequencies differed by a factor of about 20 between pH 6 and 9. The killing effect of NTG also increased with pH (Fig. 2).

As pointed out by Delić, Hopwood, and Friend (4), the measurement of mutagenesis after a single period of treatment can give a spurious pH optimum. We therefore measured the frequencies of Arg+ revertants at pH 7 and 9 after different times of treatment (Fig. 3). The initial rate of mutagenesis was higher at pH 9 than at pH 7, and the proportion of Arg+ revertants remained higher at pH 9 than at pH 7 over the whole course of the experiment (60 min).

To test whether the conditions established for Arg+ reversion appeared to be optimal also for
the induction of auxotrophic mutants in *G. tibisci*, the wild type was treated, and auxotrophic mutants were isolated by plating on nutrient agar and replica-plating to minimal medium. The results (Table 1) show that NTG treatment in pH 9 buffer gave twice as many auxotrophic mutants as at pH 7. With the procedure described in Table 1, at 1,000 μg of NTG per ml, the yield of induced auxotrophic mutants was 49.6%.

Our results with *G. tibisci* showed a pH-dependent NTG mutagenesis similar to that in streptomycetes (4). Preliminary results in *Aspergillus niger* (J. Beljak and R. Valinger, personal communication) suggest the same phenomenon in a fungus. Since decomposition of NTG increases with pH above 5 (4), a higher concentration of a mutagenic decomposition product (presumably diazomethane; 2a) is probably the reason for higher mutagenesis. The main reason for low mutant frequencies in some reports (9) may be slow release of the mutagenic product at acid or neutral pH.

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**LITERATURE CITED**


