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The Mutagenic Action of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine on *Coprinus lagopus*

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SUMMARY

Optimal conditions, defined by the use of a known auxotrophic strain, are described for the mutagenesis of *Coprinus lagopus* oidia by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG), which is a very effective mutagen for bacteria and yeast. The oidia were best treated in phosphate buffer (pH 6·8) with a concentration of 15 μg. NTG/ml. for about 70 min. The yield of auxotrophs was considerably less than might be expected from a comparison of other systems in which NTG has been used as a mutagen.

INTRODUCTION

N-Methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) has been widely used to induce mutations in bacteria. It has proved highly effective, so much so that it has been suggested to be the most potent chemical mutagen yet discovered (Adelberg, Mandel & Chen, 1965). Adelberg *et al.* (1965) found that mutations to valine resistance and to auxotrophy occurred at high frequency (up to 42·5 % auxotrophs) after exposure of *Escherichia coli* to NTG under conditions such that about 5 % of the treated bacteria remained viable. NTG has also proved a very effective mutagen for yeast, though the results are less dramatic than with *E. coli*. Megnet (1965) with *Schizosaccharomyces pombe* found that, without selection, NTG-induced auxotrophs increased to a maximum frequency of about 8 % at 20 % survival. On the other hand, Nordström (1967) obtained up to 50 % petite mutants among survivors of *Saccharomyces cerevisiae* after NTG treatment.

To date the vast majority of auxotrophs obtained in *Coprinus lagopus* have been isolated following exposure to ultraviolet (u.v.) radiation. The reported successes in the use of NTG as a mutagen in other organisms prompted the experiments reported below. It can be concluded that, under the conditions tested, NTG was no more advantageous than u.v. radiation in inducing mutants of *C. lagopus*, though it should prove to be a useful alternative.

METHODS

A strain of *Coprinus lagopus*, stock number ZMR1/66, with nutritional requirements for methionine (*met-8* allele) + nicotinic acid (*nic-4*) was used.

Complete and minimal media were made as described previously (Moore, 1968). Normal media contained 100 mM-D-glucose; sorbose media contained 100 mM-L-sorbose + 2·5 mM-D-glucose. Except where otherwise stated, nicotinic acid was added to minimal media to a final concentration of 2 μg./ml.; where required methionine was added to a final concentration of 100 μg./ml.

NTG treatment was done on oidial suspensions prepared from slope cultures growing on normal complete medium (Moore, 1968). Oidia were suspended in a buffered solution (pH 6·8) which was one of the stock medium constituents. In addition to the buffer salts (10 mM-KH_2PO_4 + 10 mM-Na_2HPO_4) this solution contained 3·3 mM-ammonium tartrate, 2 mM-Na_2SO_4 and 1×10^{-4}% (w/v) thiamine hydrochloride. Freshly harvested oidia were centrifuged and the pellet washed twice with buffer solution before being suspended in fresh buffer.

NTG was obtained from Koch–Light Laboratories Ltd., Colnbrook, Bucks., England. Solutions were made in sterile buffer immediately before use, and used without further sterilization.

All incubations and treatments were made at 37°; NTG solutions and prepared oidial suspensions were equilibrated to this temperature before mixing. The final concentration of viable oidia treated varied from 2 to 8×10^6 in 10 ml.

Treatment was terminated by filtration through a sterile Oxoid membrane filter (5 cm. size, standard grade). Oidia retained by the filter were washed with 50 ml. cold sterile buffer and then the filter was transferred to a sterile 1 oz. universal bottle containing 10 ml. buffer. Oidia were resuspended by vigorous hand shaking and the filter membrane removed. This technique enabled the rapid and complete removal of extracellular NTG. The filtration step did not significantly affect the concentration of the oidial suspension.

The filtered suspension was analysed for both survival and methionine independence. Survival was measured by plating appropriately diluted samples on sorbose complete medium. Colonies were counted after about 48 hr incubation. To determine the production of methionine-independent mutants from the methionine-dependent parent strain 1 ml. quantities of the undiluted post-treatment suspension were added to small tubes containing 2 ml. melted water agar (1 %, w/v) at 45°. After mixing the contents of the tube were poured over the surface of a plate of sorbose minimal agar. Colonies were counted after 48 to 60 hr incubation. The frequency of spontaneous methionine-independent mutants varied from 3·4 to 28×10^{-6}.

RESULTS AND DISCUSSION

Effect of different NTG concentrations. The numbers in Table 1 were derived from experiments in which oidia were exposed to NTG for a standard period of 30 min. NTG clearly exerted both toxic and mutagenic effects on *Coprinus lagopus*. This organism is evidently much more sensitive to the toxic effects of NTG than either *Schizosaccharomyces pombe* or *Escherichia coli*. It is not possible to make a precise comparison from the published data. However, with a 30 min. treatment time only 39 % of *E. coli* organisms were killed by 300 μg. NTG/ml. (Adelberg *et al.* 1965), while 80 % of treated organisms of *S. pombe* were killed by exposure to 2 mg. NTG/ml., again for 30 min. (Megnet, 1965). Very approximately, therefore, *C. lagopus* appears to be 100 times more sensitive than *E. coli*, and 200 times more sensitive than *S. pombe* to the killing effects of NTG under comparable conditions.

Effect of time of exposure to NTG. 15 μg. NTG/ml. (about 0·1 mM) was chosen as a standard concentration for further experiments. The effect of time of exposure on survival and frequency of methionine-independent mutants was determined for this concentration. Results representative of the four experiments performed are presented

in Table 2. It is clear that exposure to 15 μg. NTG/ml. for between 50 and 70 min. gave the best balance between toxicity and mutagenicity of NTG.

Effect of the suspending medium. Adelberg *et al.* (1965) and Nordström (1967) reported that NTG showed a much greater toxicity towards *Escherichia coli* and *Saccharomyces cerevisiae* when the organisms were treated in media which permitted

Table 1. *Survival and mutagenesis in Coprinus lagopus as a function of NTG concentration*

NTG concentration (μg./ml.) (30 min. exposure)	Survival (%)	Methionine-independent mutants/10^8 survivors
0	100	0·003
1	87	0·007
2	92	0·024
3	66	0·026
5	53	0·031
10	36	0·063
15	16	0·107
30	4	Not scored

Table 2. *Coprinus lagopus. Survival and mutagenesis as a function of time*

Duration of exposure to 15 μg. NTG/ml. (min.)	Survival (%)	Methionine-independent mutants/10^8 survivors
5	74	0·11
10	80	0·07
15	33	0·13
25	16	0·29
40	7	0·26
55	3	0·84
70	1·7	0·89
85	0·6	0·68

Table 3. *Coprinus lagopus. Survival as a function of the suspending medium*

Duration of exposure to 15 μg. NTG/ml. (min.)	Survival (%)		Minimal medium + methionine + nicotinic acid
	Buffer	Minimal medium	
5	86	58	40
10	73	28	12
15	38	13	5

growth than when they were exposed to the agent in buffer. A similar effect was observed with *Coprinus lagopus* strain ZMR1/66 (Table 3). Adelberg *et al.* (1965) found that the fraction of mutants among survivors was the same whether the organisms were treated in buffer or in growth-permitting medium. Though no such comparison has been made here, it is likely that a similar condition applies. In any case the use of buffer as the suspending medium is obligatory when the wild-type organism is to be treated.

Effect of NTG on the wild type strain BC9/66 of Coprinus lagopus. Experiments with

the auxotrophic strain ZMR1/66 established conditions for NTG treatment which appeared likely to be optimal for the production of mutations in wild-type oidia. The response of wild-type oidia to exposure for various times to 15 μg. NTG/ml. in buffer is shown in Table 4. The survival of BC9/66 oidia is very similar to the survival pattern of ZMR1/66 (Table 2), suggesting that mutagenic conditions shown to be optimal for ZMR1/66 most likely also applied to BC9/66. Accordingly survivors from an experiment in which BC9/66 oidia were exposed in buffer to 15 μg. NTG/ml. for 70 min. (1·85% survival) were examined and mutants isolated; 575 survivors were isolated individually for examination. Of this number three proved to be auxotrophic and seven were isolated as reliable morphological variants. One of these latter showed the 'purple' phenotype described by Morgan (1966). This result can be compared with an experiment in which BC9/66 oidia were u.v.-irradiated. Survival was 4·6% and 1050 survivors were isolated for examination; five auxotrophs were recovered. The yields of auxotrophs from these two experiments were thus approximately the same.

Table 4. *Coprinus lagopus. Survival of wild type (BC9/66) oidia as a function of time*

Duration of exposure to 15 μg. NTG/ml. (min.)	Survival (%)
10	68
20	28
40	7
70	2

Table 5. *Coprinus lagopus. Survival of wild-type oidia following incubation in minimal medium*

Duration of pre-incubation in minimal medium (hr)	Survival (%)	Auxotrophs among 250 survivors tested
0	2·6	2
1	1·9	1
3	9·8	2
5	19·1	Not tested
7	27·7	Not tested
9	60·5	Not tested
11	84·1	Not tested

Oidia were separated by filtration from the minimal medium, washed rapidly with buffer and then suspended in buffer + NTG (15 μg./ml.) for 70 min.

Incubation of BC9/66 oidia in liquid minimal medium before exposure to NTG in buffer did not lead to an increased yield of auxotrophs (Table 5). It appears that the over-all effects of the two mutagens u.v.-radiation and NTG were similar and that, at least under the conditions tested, NTG was no more effective in inducing mutations of *Coprinus lagopus* than was u.v.-radiation.

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