How to use chemical mutagens for mutagenesis.

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Background

During the 1970s, de Serres and his colleagues (Brockman *et al*. 1984) tested many chemicals for mutagenic activity in *Neurospora crassa* by using a screen for colored *ad-3* mutants as a forward mutation detection system. These mutants accumulate purple pigments in the vacuoles of the mycelium in accord with their adenine requirement (de Serres and Malling 1971). The following chemicals were confirmed as mutagens and have been used for mutagenesis of Neurospora: Mitomycin C (MMC), N-methyl-N-nitrosourea (MNU), nitrous acid (NA), diepoxybutane (DEB), 1, 2, 7, 8- diepoxyoctane (DEO), ethyl methane sulfonate (EMS), methyl methane sulfonate (MMS), N-methyl- N'-nitro-N-nitrosoguanidine (MNNG), 4-nitroquinoline 1-oxide (4-NQO), 2-methyloxy-6-chloro-9(3-[ethyl-2-chloroethyl]-aminopropylamino)-acridinedihydrochloride (ICR-170), 2-amino purine (2AP), and hydroxylamine (HA). Table 1 summarizes the characteristic types of DNA damage and resulting mutations induced by these chemicals.

Table 1.

Chemical mutagen	Type of DNA lesion	Major mutation type
4-NQO DEB	DNA adducts	Base-pair substitution
ICR-170	Intercalation	Frameshift
MMC DEO	Interstrand cross-linking	Deletion
MNNG EMS MNU	Alkylation	Base-pair substitution
MMS	Alkylation/Strand breaks	Several different types
NA HA	Modification of bases	Base-pair substitution
2AP	Base analog	Base-pair substitution

Procedures

1. Treatment by alkylating agents; MNNG or MMS (Malling and de Serres 1970; Inoue and Schroeder 1988.

A wild-type conidial suspension ($2x10^6$/ml) is prepared in 0.067 M phosphate buffer (pH 7.0)

and kept at 4ºC. Just before treatment 20ml of the suspension is pre-warmed at 30ºC with mild shaking. Chemical mutagens are added at a final concentration of 25μM for MNNG or 1.5μl/ml for MMS. After a 2 hr treatment, conidia are collected by centrifugation and washed three times with a solution of Fries minimal medium salts (pH 8.0) containing 0.1% sodium thiosulfate. This MNNG treatment results in 50-70% survival and 100-150 *ad-3* mutants per 10^6 survivors. The MMS treatment results in 30-50% survival and 50-80 *ad-3* mutants per 10^6 survivors.

2. Treatment by ICR-170 (Inoue et al. 1981; Whong 1979).
ICR-170 is sensitive to visible light so treatments should be done under a safety light (yellow or red) or in the dark to avoid photoinactivation. Mutagenesis with ICR-170 is conducted essentially as described above. Conidia suspended in the 0.067 M phosphate buffer are treated with 15μM ICR-170 for 2 hrs. This treatment results in about 80% survival and 100-200 *ad-3* mutants per 10^6 survivors. Substantial mutagenic activities can be obtained at lower concentrations of ICR-170 when the treatment is conducted in distilled water and bubbled with N_2.

3. Treatment by 4-NQO (Matter, Ong and de Serres 1972)
This chemical is highly toxic, and stock solutions are prepared in 95% ethanol. Mutagenesis treatments are conducted as described above but with 0.2μM 4-NQO. This treatment results in about 10-30% survival and 100-200 *ad-3* mutants per 10^6 survivors.

4) Treatment by DEO. (Ong and de Serres 1972)
Mutagenesis treatments are conducted as described above. When DEO is used at 75mM or 100mM, survival of cells is 30% and 10%, and mutation frequency is about $5x10^{-7}$ and $8x10^{-7}$, respectively.

In all experiments, treatment time and concentration of chemicals will be variable depending on your purpose. Mutation frequency at the *ad-3* loci described here serves as one example.

Reference

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