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A method of NTG mutagenesis of N. crassa.

The following method of N-methyl-N′-nitro-N-nitroroguclnidine mutagenesis has been used to yield on auxotrophic mutant production rate as high as 17%. This method is particularly useful where other methods of mutagenesis, such as UV light, ore unavailable or unadvisable. The wild-type strain STA4, St. Lawrence standard 74A was used for all tests. The NTG is first prepared by dissolving it in distilled water to give a concentration of 3 mg/ml. NTG is slightly insoluble in water and the solution may hove to be warmed slightly to oid in dissolving. Freshly prepared solution will give the best results, as NTG deteriorates with age. (At 30 days it is less than 50% as active as fresh). A conidiol suspension is then mode by washing the conidio from a slant culture using sterile distilled water, filtering it through eight layers of sterile gauze, and adjusting it to a final concentration of 1 x 10^6 conidio per cc.

Two ml of the NTG solution is added to 100 ml of the 1 x 10^6 conidiol suspension, giving on NTG concentration of 0.06mg/ml of medium. This is allowed to stand at room temperature for 30 minutes with periodic agitation. A sample is then removed ond diluted to a concentration of 1 x 10^3conidia/cc. One ml ond 0. 1 ml of this concentration are both plated and spread on complete sorbose medium. The plates are then incubated at 25°C for two to three days. When colonies become apparent, replica plating to minimal sorbose may be utilized to rapidly isolate the biochemical mutants. Mutants are then characterized and crossed with a wild-type strain to verify that they are truly mutants.

Table 1. Effect of varying NTG concentration on survival and mutant yield.

NTG Concentration	0.06 mg/ml medium						0. 12 mg/ml medium					
Exposure time (min)	5	15	30	45	60	90	5	15	30	45	60	90
% Survival	45	27	32	22	6	5	35	29	23	19	4	4
% Mutant yield			17	9					2	8		

The above concentrations ond recommended times were arrived at through several tests. The results of there tests are combined in Table 1. We began the tests using on NTG concentration of 0.03 mg/ml medium. This concentration produced mutants, but required from 60 to 90 minutes of exposure time. The 0.06 mg/ml concentration was chosen over the lower one because it allowed completing the work in a much shorter time. The 0. 12 mg/ml concentration, while time-raving, doer not reduce the survival percentage as much as might be expected, although it drastically reduces the percent mutant yield. The 30 minute time of exposure was chosen because of the high mutant yield and the convenience of the shorter time span. Additional tests showed no increase in survival as a result of washing the NTG from the conidiol suspension with sterile distilled water prior to plating.

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