Sullivan, J. L. and A. G. DeBusk. Method for specific selection of temperature-sensitive mutants.

The inositol-less death technique for selection of auxotrophic mutants (Lester ond Gross 1959 Science 129:572) has been adopted for specific selection of temperature-sensitive mutants by using the temperature-sensitive, inositol-requiring, double mutant inos; ylo-1 (83201(t); Y30539y) as the parental strain. This strain requires inositol for growth at 35°C but not for growth at 25°C. It is killed by incubation in on inositol-less medium at 35°C but grows well on the same medium at the lower temperature.

A suspension of inos; ylo-1 conidio is mutagenized, plated on sorbose minimal medium and incubated at 35°C for 36-48 hrs. The plater ore then shifted to an incubation temperature of 25°C. Colonies may be picked 2-5 days after the temperature shift. Isolates may then be tested for failure to grow on inositol supplemented minimal medium at 35°C. This method provider a strong selection for temperature-sensitive mutants, which will not begin to grow on minimal medium at 35°C (and therefore will not be killed in the absence of inositol) but which con doer grow on minimal medium at 25°C. The parental rtroin is killed by incubation at 35°C in the absence of inositol, and auxotrophic mutants that ore not tempemture-sensitive should not be able to grow on minimal medium at either temperature and therefore should not develop on the plates. Overlaying of plates with supplementary inositol-comtaining medium is unnecessary since inos; ylo-1 doer not require inositol at 25°C.

Preliminary screening of 65 isolates picked indiscriminately from plates incubated for 36 ond 48 hours at 35°C, and then at 25°C until colonies formed, in one experiment indicates that 7 ore tempemture-sensitive auxotrophs, 10 are temperature-sensitive unknowns and 6 are morphological variants, which gives a tempemture-sensitive mutant frequency of 0.26. (Survival frequency after UV irradiation was 0.45: number of viable conidio per plate was approximately 4.5 x 1 0^5; survival frequency after 36 hours incubation at 35°C was 9 x 10^5, and after 48 hours, 2 x 10^5. This temperature-sensitive version of the inositol-less deoth technique simplifies ond shortens the old procedure by eliminating the agar overlaying step and greatly reducing the inaitol-less incubation time. - - - Genetics Laboratories, Department of Biological Science, The Florida State University, Tallahassee, Florida 32306.