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Enrichment for cold-sensitive mutants by tritium suicide

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Abstract

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Tritium suicide is defined as the death of cells caused by the decoy of tritium that hos become incorporated into their macromolecular components. It has been used as on effective enrichment procedure for the isolation of temperature-sensitive and auxotrophic mutants of yeast and E. coli and for the isolation of auxotrophic mutants of Aspergillus flavus. The advantage of tritium suicide over other enrichment procedures is that it can be mode relatively specific by the use of particular tritiated compounds. The following is a description of a method devised to select for cold-sensitive mutants of N. crassa using tritiated uridine.

Conidia of wild-type strain 74A were mutagenized with 15 μg nitrosoguanidine ml^{-1} for 3 h at 25 C with shaking. Under there conditions a 77% killing was obtained. Conidia, washed free of the mutagen, were suspended in Vogel's minimal medium to a final concentration of 1.4×10^7 viable conidia ml^{-1}. Two 5 ml sampler were incubated with shaking at 25 C in 50 ml Delong flasks for 1.5 h and then equilibrated to 10 C. [5-^{3}H] uridine (Schwarz-Mann, specific activity 8 Ci $mmol^{-1}$) was added to one sample to a final concentration of 40 μCi ml^{-1}. The other (control) culture received no label. The flasks were incubated for 24 h at 10 C with shaking, after which time the conidio were washed twice with water, resuspended in 5 ml of Vogel's minimal salts and then stored at 4 C. Viability in both the experimental and control cultures was determined at this time (zero time) and at various timer thereafter.

After 19 days of storage, viability of the control culture was 86% that of the zero time value, whereas viability in the experimental culture hod dropped to 7% of the zero time control value. At this time samples from both cultures were plated on Vogel's complete medium and putative cold-sensitive mutants rescued as described by Schlitt and Russell (1974 J. Bacteriol. 120: 666). No cold-sensitive mutants were obtained from the control culture, whereas 86 mutant strains were isolated from on equivalent plating of the experimental culture. This plating represented only a small fraction of the total culture.

Thus tritium suicide appears to be an effective enrichment procedure in N. crassa, also. The use of tritiated uridine as the tritium source should hove enriched for mutants with conditional blocks in RNA metabolism, and this is currently being investigated. A further advantage of this method over filtration enrichment as a means of selecting for cold-sensitive mutants is that the risk of contamination appears to be reduced considerably. - - - Biology Department, Reed College, Portland, Oregon 97202.