

Specific selection of temperature-sensitive mutants

J. L. Sullivan
Florida State University

A. G. DeBusk
Florida State University

Follow this and additional works at: <http://newprairiepress.org/fgr>

Recommended Citation

Sullivan, J. L., and A.G. DeBusk (1971) "Specific selection of temperature-sensitive mutants," *Fungal Genetics Reports*: Vol. 18, Article 18. <https://doi.org/10.4148/1941-4765.1897>

This Technical Note is brought to you for free and open access by New Prairie Press. It has been accepted for inclusion in Fungal Genetics Reports by an authorized administrator of New Prairie Press. For more information, please contact cads@k-state.edu.

Specific selection of temperature-sensitive mutants

Abstract

Specific selection of temperature-sensitive mutants

Creative Commons License



This work is licensed under a [Creative Commons Attribution-Share Alike 4.0 License](https://creativecommons.org/licenses/by-sa/4.0/).

Sullivan, J. L. and A. G. DeBusk. Method for

specific selection of **temperature-sensitive mutants.**

(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 grow on minimal medium at 25°C. The parental rtrouin 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-containing 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 4.5×10^5 ; survival frequency after 36 hours incubation at 35°C was 9×10^5 , and after 48 hours, 2×10^5 . This temperature-sensitive version of the inositol-less death 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.