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Temperature-sensitive mutant strains for isolation of additional mutants of a given site

S. Brody

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Abstract
Use of temperature-sensitive strains

Brody, Sf temperature-sensitive mutant strains for isolation of additional mutants at a given site.

For purposes of correlation with the results of studies on N. crassa strain col-2 (Y5331), it was desirable to isolate additional strains altered-e col-2 locus ond to examine the properties of their glucose-6-P dehydrogenases. However, the isolation of a particular colonial strain would involve numerous mutant hunts and extensive mapping and/or biochemical screening since the colonial phenotype can be due to mutation at many different genes (40 at least). The selection and isolation of temperature-sensitive revertants circumvented these problems. The rationale for this approach is as follows: revertants from a presumed point mutation in a structural gene may be due to mutations at the original site, elsewhere in the same gene, or at another locus. Quite often compensatory mutations at another site in the gene, the so-called second-rite revertants, lead to the production of temperature-sensitive proteins. Therefore, some of the temperature-sensitive revertants may be second-site revertants. Approximately 40 wild-type revertants were isolated at 25°C and 4 of these were found to be colonial at 35°C. One of these temperature-sensitive strains was the strain desired; i.e., it had a temperature-sensitive glucose-6-P dehydrogenase.

Another temperature-sensitive revertant, which proved to be a temperature-sensitive suppressor strain, was helpful in that it was used as a source of conidia for the inositol-less death mutant selection technique. Selection of mutants in most colonial strains is difficult due to the lock of conidiation. However, this difficulty is bypassed by harvesting conidia from this suppressor strain grown at 25°C and then performing the mutant selection at 35°. Selection of certain mutants in colonial strains may be advantageous, since the altered metabolism of the colonial strains might not allow the growth of certain "leaky" mutants during inositol deprivation. * * * Rockefeller University, New York, New York. 10021.