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

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C. Notes: 1. Lyophilized material can be used only if the culture has been lyophilized immediately after harvesting. Frozen and thawed cultures which were extracted, or lyophilized and then extracted, gave variable results, possibly due to glycogen breakdown. Also aerobic cultures grown to the point of carbon-source exhaustion had lower levels of glucose-6-P. Therefore, only actively growing cultures can be used for the assay.

2. Extraction by cold 1 M HClO4 (and subsequent neutralization with cold KOH) gave comparable results to the ethanol extractions, as did extraction with cold 10% TCA. In both cases, the glucose-6-P could be assayed properly only after inhibitors were removed by chromatography.

3. Extracts are not concentrated to dryness since phosphorylated compounds occasionally adhere to glass surfaces.

4. Almost complete clarification can be obtained by 100,000 x g for 90 minutes.

5. Glucose-6-P in extracts tends to trail somewhat, particularly on paper which has not been treated with EDTA.

6. Core must be taken to avoid small bits of paper in the eluate as they subsequently interfere with the assay.

7. Other sources occasionally contained significant amounts of 6-phosphogluconic acid dehydrogenase (thereby doubling all values obtained), as well or traces of isomerasers.

8. It is not known in what form the glucose-6-P is isolated, so a molecular weight of 340 (i.e., Na2glucose-6-P.2H2O) was assumed.  

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Brody, S. 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 at col-2 locus and 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 conidial death for the selection technique. Selection of mutants in most colonial strains is difficult due to the lack of conidiation. However, this difficulty is bypassed by harvesting conidia from this suppressor strain grown at 25°C and then performing the selection at 35°C. 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.  

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Pall, M. L. The use of cycloheximide as an inhibitor of protein synthesis in Neurospora.  

The antibiotic cycloheximide (Actidione) has been reported to be an inhibitor of protein synthesis in some fungi, higher animals, and higher plants. It is shown here to be an effective inhibitor of protein synthesis in Neurospora crassa.

Wild-type strain 69-1113a was grown for 2 days at 25°C in 20 ml of Vogel's Medium N + 2% sucrose. Cycloheximide was added and the cultures gently shaken on a reciprocal shaker for one hour. They were then given a 12-minute pulse of 0.5 µC 14C L-lysine. The mycelial pads were fractionated according to the procedure of Roberts, et al. (1955 Carnegie Inst. Washington Publ. 607: 207).

<table>
<thead>
<tr>
<th>Concentration of cycloheximide (µg/ml Vogel's medium)</th>
<th>Percent of counts taken up incorporated into protein</th>
<th>Percent inhibition of protein synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>63.3 %</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4.44 %</td>
<td>93.0 %</td>
</tr>
<tr>
<td>10</td>
<td>1.11 %</td>
<td>98.2 %</td>
</tr>
</tbody>
</table>

Cycloheximide has also been used to study the inducible enzyme tyrosinase. When ethionine is added, there is a lag period before synthesis starts, followed by a period of rapid synthesis. When cycloheximide (20 µg/ml) is added during the lag period, no activity develops. When it is added during the synthetic period, the activity remains at the level reached at the time of addition of the cycloheximide. These results are consistent with the idea that the tyrosinase is de novo protein synthesis and the cycloheximide inhibits any further synthesis of the enzyme.  

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