have shrunk the slant somewhat, but not so as to dessicate the spores. Only if isolation is delayed until the agar has shrunk to half or less its original volume do we take steps to assure that ascospores are hydrated. With crosses in small tubes (10 x 75 mm), drying out occurs quickly and rehydration is more likely to be necessary.

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Westrum, F. M. and N. V. Vigfusson.

A method of NTG mutagenesis of N. crassa.

The following method of N-methyl-N'-nitro-N-nitroguanidine mutagenesis has been used to yield on auxotrophic mutant production rate of 17%. This method is particularly useful where other methods of mutagenesis, such as UV light, are unavailable or unadvisable. The wild-type strain STA4, St. Lawrence standard 74A was used for all tests. The NTG is first prepared by dissolving it in distilled water to give a concentration of 3 mg/ml. NTG is slightly insoluble in water and the solution may have to be warmed slightly to aid in dissolving. Freshly prepared solution will give the best results, as NTG deteriorates with age. (At 30 days it is less than 50% as active as fresh). A conidial suspension is then made by washing the conidium from a slant culture using sterile distilled water, filtering it through eight layers of sterile gauze, and adjusting it to a final concentration of 1 x 10⁶ conidio per cc.

Two ml of the NTG solution is added to 100 ml of the 1 x 10⁶ conidial suspension, giving on NTG concentration of 0.06 mg/ml of medium. This is allowed to stand at room temperature for 30 minutes with periodic agitation. A sample is then removed and diluted to a concentration of 1 x 10³ conidia/cc. One ml of this concentration is then plated on complete sorbose medium. The plates are then incubated at 25°C for two to three days. When colonies become apparent, replica plating to minimal sorbose may be utilized to rapidly isolate the biochemical mutants. Mutants are then characterized and crossed with a wild-type strain to verify that they are truly mutants.

Table 1. Effect of varying NTG concentration on survival and mutant yield.

<table>
<thead>
<tr>
<th>NTG Concentration</th>
<th>0.06 mg/ml medium</th>
<th>0.12 mg/ml medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure time (min)</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>% Survival</td>
<td>45</td>
<td>27</td>
</tr>
<tr>
<td>% Mutant yield</td>
<td>17</td>
<td>9</td>
</tr>
</tbody>
</table>

shorter time. The 0.12 mg/ml concentration, while time-raking, does not reduce the survival percentage as much as might be expected, although it drastically reduces the percent mutant yield. The 30 minute time of mutant yield and the convenience of the shorter time span. Additional tests showed no effect of the NTG from the conidial suspension with sterile distilled water prior to plating.

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Kulaev, I. S. and V. I. Melgunov. Determination of phosphorus in N. crassa extracts.

A method of total phosphorus determination in Neurospora extracts has been described by Hedmon (1969 Neurospora News 14: 10), who suggested the use of a method for total orthophosphate content estimation. However, like all other colorimetric methods of phosphate determination in the aqueous phase, this one seems to be liable to error arising from some disturbing factors (see Berenblum and Chain 1938 Biochem. J. 26: 295). Besides which, the time of interaction between molybdate and phosphorous compounds in solution was very long (5-10 min). Thus, the total orthophosphate content will be overestimated because of the well-known catalytic effect of molybdate on the hydrolysis of organic phosphates. Therefore, we wish to turn Neurosporologists' attention to another, more advantageous procedure of phosphate determination.

The method adopted in our laboratory is based mainly on Weil-Malherbe and Green (1951 Biochem. J. 49: 286) and Martin and Doty (1949 Analyt. Chem. 21: 965) modifications of the extraction method of phosphate estimation introduced by Berenblum and Chain (1938, ibid.). The solutions used were: (1) mixture of isobutanol-benzene (1:1, v/v). (2) 5% ammonium molybdate in 4N H₂SO₄ prepared fresh daily by dilution of stock solution of 10% ammonium molybdate in 8N H₂SO₄. (3) Stock solution of stannous chloride; 10 g SnCl₂ dissolved in 25 ml conc. HCl, kept in a brown glass-stoppered bottle at 0°C. (4) Dilute stannous chloride solution; 0.25 ml conc. solution diluted to 10 ml with 1N H₂SO₄ (must be made up fresh when required). (5) Acid ethanol; 10 ml conc. H₂SO₄ + 490 ml absolute ethanol.

Procedure: If the solution to be tested is strongly acid or alkaline, it must first be neutralized to pH 7-8 with NaOH or HCl. Then add 6 ml isobutanol-benzene mixture and 1 ml 5% ammonium molybdate in 4N H₂SO₄ to the test solution made up to 5 ml in a glass-stoppered test tube. Shake it immediately for 15 sec. With a fine-tipped pipette connected to suction flask, discard the aqueous bottom layer as completely as possible. Then add a pinch of anhydrous Na₂SO₄ to the test tube and shake it until the extract is cleared of any emulsified droplets. By means of a syringe pipette, withdraw 2 ml and transfer to a second test tube. Add 2 ml acid ethanol, 0.1 ml dilute SnCl₂ solution and mix by shaking. After 10 min, the intensity of blue color may be determine...
mined either by an ordinary colorimeter with a red filter or by means of spectrophotometry at 650 nm in cuvettes of 1.0 cm path length. Construct a calibration curve in the usual way. The stock phosphate solution required for comparison is prepared as follows: 2.193 g KH₂PO₄ in 500 ml water (= 1 mg P/ml).

Under the conditions described, linearity is observed between absorbance and phosphorous content over the range of 1-25 pg. 21.2 μg of Phosphorus gives an optical density of 1.000 ± 0.010. The most reliable results are obtained in the range of 1-10 μg. The phosphate determination can also be utilized to estimate the total phosphorous content and the content of acid-labile phosphates. The sum of labile phosphates and orthophosphate is determined in a cooled neutral hydrolysate of the rompule after 10 min hydrolysis with an equal volume of 2 N HCl in a boiling water bath.

For the determination of total phosphorous the rompule content must be incinerated by the addition of 0.2-0.3 ml of 57% HClO₄ and the subsequent heating of the rompule on a special electric stove equipped with a contact thermometer and a duraluminum disc with rockets (about 50 in number) for the test tube. For the first 1-2 hours, the heating is carried out at 110-120°C, until the water has completely evaporated. Then the temperature is raised to 170-180°C and incineration proceeds to obtain a fully colorless solution. The incinerated sample is made up to 1-2 ml, approximately, by adding water and the test tube is heated in a boiling water bath for 10 min to hydrolyze pyrophosphates formed during incineration. The determination of phosphate in the neutralized rompule is carried out as usual.

The advantages of this method are related to the discarding of the aqueous layer, the absence of the non-specific development of blue color in the control and the reduced contact between the molybdate reagent and the labile phosphate bonds (15 sec), all of which were accurately stated in the papers of the authors cited above.

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Ogata, W.N. Preservation of aconidial-aconidial Neurospora cultures using anhydrous silica gel.

Davison Chemical Corporation, Baltimore, MD, refrigerator grade PA-400), which is a less granular and better grade of silica gel from that described in NN §1, is saturated with a prepared sterile solution of 1:1 concentration of each medium: Bacto corn meal agar, with dextrose (Difco #0014-01), Bacto Neurospora culture agar, (Difco #0321-5), Bacto Neurospora minimal (Difco #0817-01), and reconstituted powdered milk (7 grams/100 ml distilled water).

A sample of the culture is transferred onto the soft agar-silica gel slant and allowed to grow in an incubator at 30-32°C (25°C for temperature sensitive mutants) for 3-5 days. The culture tube (FGSC pre-freezes the culture tube in crushed dry ice) is placed in a vacuum desiccator containing a dish of powdered P₂O₅ as a desiccant and evacuated with a vacuum pump. The desiccator is stored in a deep freeze overnight to permit desiccation. If the tube is not dry on inspection, it may be necessary to repeat the step. When the culture tube has dried, a layer of sterile anhydrous silica gel is added to the tube and sealed with a sterile screw cap for storage. Submerge the cap completely in molten paraffin for indefinite storage. The extra layer of silica gel is added to take up any moisture that might seep into the tube during storage. The dehydrated culture tubes are stored in a plastic container to which a layer of talc-talc silica gel has been added to absorb any moisture seeping into the storage container. Cultures preserved by this method have been tested after 3-15 months of storage and of the 57 cultures tested, viability was 96%.

Sampler from the above culture tube during the growth stage have been used for lyophilization with very reliable results.

This work was supported by National Science Foundation Grant No. 30487. - - - Fungal Genetics Stock Center, California State University, Humboldt, Arcata, California 95521.